CFX96™ and CFX96 Deep Well Real-Time PCR Detection Systems

Instruction Manual

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CE IVD

BIO-RAD

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NOTICE REGARDING BIO-RAD THERMAL CYCLERS AND REAL-TIME SYSTEMS

This product is covered by one or more of the following U.S. patents or their foreign counterparts owned by Eppendorf AG: U.S. Patent Numbers 6,767,512 and 7,074,367.

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Instruction Manual Information

This manual contains information on how to safely set up and operate the CFX96™ real-time PCR detection system that carries the CE-IVD mark (catalog number 1855095–IVD) as well as information on how to safely set up and operate the CFX96™ Deep Well real-time PCR detection system (catalog number 1854095–IVD). **These two systems will be referred to as the CFX system in this instruction manual.** Additional information on CFX Manager™ software can be found in the CFX96 and CFX96 Deep Well Instruction Manual for life science research (catalog numbers 1855095, 1844095).

Writing Conventions Used in this Manual

The manual uses the writing conventions listed in Table 1.

Table 1. Conventions used in this manual.

Convention	Meaning
TIP:	Provides helpful information and instructions, including information explained in further detail elsewhere in this manual.
NOTE:	Provides important information, including information explained in further detail elsewhere in this manual.
WARNING!	Explains very important information about something that might damage the researcher, damage an instrument, or cause data loss.
X > Y	Select X and then select Y from a toolbar, menu or software window.

For information about safety labels used in this manual and on the CFX system, see, “Safety and Regulatory Compliance” on page iii.

Bio-Rad Resources

Table 2 lists Bio-Rad resources and how to locate what you need.

Table 2. Bio-Rad resources.

Resource	How to Contact
Local Bio-Rad Laboratories representatives	Find local information and contacts on the Bio-Rad website by selecting your country on the home page (www.bio-rad.com). Find the nearest international office listed on the back of this manual.
Technical notes and literature	Go to the Bio-Rad website (**www.bio-rad.com**). Type a search term in the Search box, and select **Literature** to find links to technical notes, manuals, and other literature.
Technical specialists	Bio-Rad’s Technical Support department is staffed with experienced scientists to provide customers with practical and expert solutions. To find local technical support on the phone, contact your nearest Bio-Rad office. For technical support in the United States and Canada, call 1-800-424-6723 (toll-free phone), and select the technical support option.

Safety and Regulatory Compliance

For safe operation of the CFX system, we strongly recommend that you follow the safety specifications listed in this section and throughout this manual.

The CFX96 system is approved for use as in vitro diagnostic (IVD) medical equipment.

Safety Warning Labels

Warning labels posted on the instrument and in this manual warn you about sources of injury or harm. Refer to Table 3 to review the meaning of each safety warning label.

Table 3. Meaning of safety warning labels.

	CAUTION: Biohazard! This symbol identifies components that may become contaminated with biohazardous material.
	CAUTION: Risk of danger! This symbol identifies components that pose a risk of personal injury or damage to the instrument if improperly handled. Wherever this symbol appears, consult the manual for further information before proceeding.
	CAUTION: Hot surface! This symbol identifies components that pose a risk of personal injury due to excessive heat if improperly handled.

Instrument Safety Warnings

The warning labels shown in Table 4 also display on the instrument and refer directly to the safe use of the system.

Table 4. Instrument safety warning labels.

Icon	Meaning
	Warning about risk of harm to body or equipment. Operating the CFX system before reading this manual can constitute a personal injury hazard. For safe use, do not operate this instrument in any manner unspecified in this manual. Only qualified laboratory personnel trained in the safe use of electrical equipment should operate this instrument. Always handle all components of the system with care and with clean, dry hands.

Table 4. Instrument safety warning labels. (continued)

Icon	Meaning
	Warning about handling biohazardous materials. When handling biohazardous samples, adhere to the recommended precautions and guidelines, and comply with any local guidelines specific to your laboratory and location.
	Warning about risk of burning. A thermal cycler generates enough heat to cause serious burns. Wear safety goggles or other eye protection at all times during operation. Always allow the sample block to return to idle temperature before opening the lid and removing samples. Always allow maximum clearance to avoid accidental skin burns.
	Warning about risk of explosion. The sample blocks can become hot enough during the course of normal operation to cause liquids to boil and explode.

Safe Use Specifications and Compliance

Table 5 lists the safe use specifications for the CFX system. Shielded cables (supplied) must be used with this unit to ensure compliance with the Class A FCC limits.

Table 5. Safe use specifications.

Safe Use Requirements		Specifications
Temperature	Indoor use	Ambient temperature 15–31°C. Relative humidity maximum 80%, noncondensing
Altitude		Up to 2,000 meters above sea level

Regulatory Compliance

This instrument has been tested, and found to be in compliance with all applicable requirements of the following safety and electromagnetic standards:

- IEC 61010-1:2001 (2nd Ed.), EN61010-1:2001 (2nd Ed). Electrical Equipment For Measurement, Control, and Laboratory Use - Part 1: General Requirements
- IEC 61010-2-010:2005, EN61010-2-010:2003. Safety requirements for electrical equipment for measurement, control and laboratory use. Part 2-010: Particular requirements for laboratory equipment for the heating of materials
- IEC 61010-2-081:2001+A1, EN61010-2-081:2002+A1. Safety requirements for electrical equipment for measurement, control and laboratory use. Part 2-081: Particular requirements for automatic and semi-automatic laboratory equipment for analysis and other purposes (includes Amendment 1)
- EN 61326-1:2006 (Class A) Electrical equipment for measurement, control and laboratory use. EMC requirements, Part 1: General requirements
- EN 61010-2-101. Safety requirements for electrical equipment for measurement, control and laboratory use. Particular requirements for in vitro diagnostic (IVD) medical equipment
- EN 61326-2-6 (Class A) Electrical equipment for measurement, control and laboratory use. EMC requirements. Part 2-6. Particular requirements. In vitro diagnostic (IVD) medical equipment

This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference in which case the user will be required to correct the interference at his own expense.

Hazards

The CFX system is designed to operate safely when used in the manner prescribed by the manufacturer. If the system or any of its associated components are used in a manner not specified by the manufacturer, the inherent protection provided by the instrument may be impaired. Bio-Rad Laboratories, Inc. is not liable for any injury or damage caused by the use of this equipment in any unspecified manner, or by modifications to the instrument not performed by Bio-Rad or authorized agent. Service of the system should be performed only by Bio-Rad personnel.

Biohazards

The CFX system is a laboratory product. However, if biohazardous samples are present, adhere to the following guidelines and comply with any local guidelines specific to your laboratory and location.

General precautions

- Always wear laboratory gloves, coats, and safety glasses with side shields or goggles
- Keep your hands away from your mouth, nose and eyes
- Completely protect any cut or abrasion before working with potentially infectious materials
- Wash your hands thoroughly with soap and water after working with any potentially infectious material before leaving the laboratory
- Remove wristwatches and jewelry before working at the bench
- Store all infectious or potentially infectious material in unbreakable, leak-proof containers
- Before leaving the laboratory, remove protective clothing
- Do not use a gloved hand to write, answer the telephone, turn on a light switch, or touch anything that other people may touch without gloves
- Change gloves frequently. Remove gloves immediately when they are visibly contaminated
- Do not expose materials that cannot be properly decontaminated to potentially infectious material
- Upon completion of the operation involving biohazardous material, decontaminate the work area with an appropriate disinfectant (for example, a 1:10 dilution of household bleach)

Specific precautions

- All patient samples are a potential biohazard and should be handled accordingly using universal precautions
- No biohazardous substances are exhausted during normal operations of this instrument

Surface decontamination

WARNING! To prevent electrical shock, always turn off and unplug the instrument prior to performing decontamination procedures.

The following areas can be cleaned with any hospital-grade bactericide, virucide, or fungicide disinfectant:

- Outer lid and chassis
- Inner reaction block surface and reaction block wells

- Control panel and display

To prepare and apply the disinfectant, refer to the instructions provided by the product manufacturer. Always rinse the reaction block and reaction block wells several time with water after applying a disinfectant. Thoroughly dry the reaction block and reaction block wells after rinsing with water.

WARNING! Do not use abrasive or corrosive detergents or strong alkaline solutions. These agents can scratch surfaces and damage the reaction block, resulting in loss of precise thermal control.

Disposal of Biohazardous Material

The CFX system contains no potentially hazardous chemical materials. Dispose of the following potentially contaminated materials in accordance with laboratory local, regional and national regulations:

- Clinical samples
- Reagents
- Used reaction vessels or other consumables that may be contaminated

Chemical Hazards

The CFX system contains no potentially hazardous chemical materials.

Explosive or Flammability Hazards

The CFX system poses no uncommon hazard related to flammability or explosion when used in a proper manner as specified by Bio-Rad Laboratories.

Electrical Hazards

The CFX system poses no uncommon electrical hazard to operators if installed and operated properly without physical modification and connected to a power source of proper specification.

Transport

Before moving or shipping the C1000™ thermal cycler or optical reaction module, decontamination procedures must be performed. Always move or ship the C1000 thermal cycler chassis and optical reaction module in separate containers with the supplied packaging materials that will protect the instrument from damage. If appropriate containers cannot be found, contact your local Bio-Rad office.

Storage

The CFX system can be stored under the following conditions:

- Temperature range –20 to 60ºC
- Relative humidity maximum 80%

Disposal

The CFX system contains electrical or electrical materials; it should be disposed of as unsorted waste and must be collected separately according to the European Union Directive 2002/96/CE on waste and electronic equipment —WEEE Directive. Before disposal, contact your local Bio-Rad representative for country-specific instructions.

Table of Contents

Chapter 1. System Installation 1
Unpacking the Optical Reaction Module 1
System Requirements 1
System Overview 2
Setting Up the System 3
Installing CFX Manager Software 5
Software Files 7
Running Experiments 7

Chapter 2. CFX Manager™ Software 9
Main Software Window 9
Startup Wizard 12
Detected Instruments Pane 12
Instrument Properties Window 14

Chapter 3. Running Experiments 17
Experiment Setup Window 17
Protocol Tab 18
Plate Tab 19
Start Run Tab 19
Run Details Window 20

Chapter 4. Protocols 25
Protocol Editor Window 25
Protocol Editor Controls 27
Temperature Control Mode 30
Protocol AutoWriter 30

Chapter 5. Plates 33
Plate Editor Window 33
Select Fluorophores Window 35
Well Loading Controls 36
Experiment Settings Window 38
Well Groups Manager Window 40
Plate Spreadsheet View Window 42

Chapter 6. Stand-Alone Operation 43
C1000 Thermal Cycler 43
Creating a New Experiment 45
Exporting Data for Analysis 49
Creating a Data File 51

Chapter 7. Data Analysis Overview 53
Data Analysis Precautions 53
Data Analysis Window 54
Quantitation Tab 57
Data Analysis Settings 58
Well Selectors 60
Charts 62
Spreadsheets 62

Chapter 8. Data Analysis Windows 65
Quantitation Tab 65
Quantitation Data Tab 68
Melt Curve Tab 69
Melt Curve Data Tab 70
End Point Tab 70
Allelic Discrimination Tab 72
QC Tab 74
Run Information Tab 74
Data File Reports 75

Chapter 9. Gene Expression Analysis 79
Gene Expression 79
Plate Setup for Gene Expression Analysis 80
Gene Expression Tab 80
Experiment Settings Window 85
Gene Study 86
Gene Study Data Spreadsheet 89
Gene Study Report Window 90

Chapter 10. Users and Preferences 91
Log In or Select User 91
User Preferences Window 92
Configure Email Notification 93
User Administration 97

Chapter 11. Resources 99
LIMS Integration 99
Calibration Wizard 102
Instrument Maintenance 104
Application Log 106
Troubleshooting 106
References 108

1 System Installation

Read this chapter for information about setting up the CFX system:

- Unpacking the optical reaction module (page 1)
- System requirements (page 1)
- System overview (page 2)
- Setting up the system (page 3)
- Installing CFX Manager™ software (page 5)
- Software files (page 7)
- Running experiments (page 7)

Unpacking the Optical Reaction Module

The optical reaction module shipment includes these components:

- Optical reaction module
- USB cable
- CFX Manager software installation CD
- Instruction manual

Remove all packing materials and store them for future use. If any items are missing or damaged, contact your local Bio-Rad office.

System Requirements

To operate the CFX system, use the following power sources and cables:

- **Input power.** 100–240 VAC, 50–60 Hz
- **Indoor use.** Ambient temperature 15–31°C. Relative humidity maximum 80%, non-condensing
- **USB cable.** If the CFX system is going to be controlled by a computer via a USB cable, the cable provided by Bio-Rad is sufficiently shielded for use.

  NOTE: For a full list of the safety and compliance requirements for this instrument, see “Safety and Regulatory Compliance” on page iii.

System Overview

The CFX system includes two components:

- **Optical reaction module.** This module includes an optical system to collect fluorescent data and a thermal cycler block

  NOTE: The serial number of the CFX module is located on the back.

- **C1000™ thermal cycler base.** The C1000 base includes a user interface to control the system when running in stand-alone mode and a power button and ports (both on back panel) to connect to a computer

Figure 1. Front view of the CFX system.

When open, the CFX system includes the features shown in Figure 2.

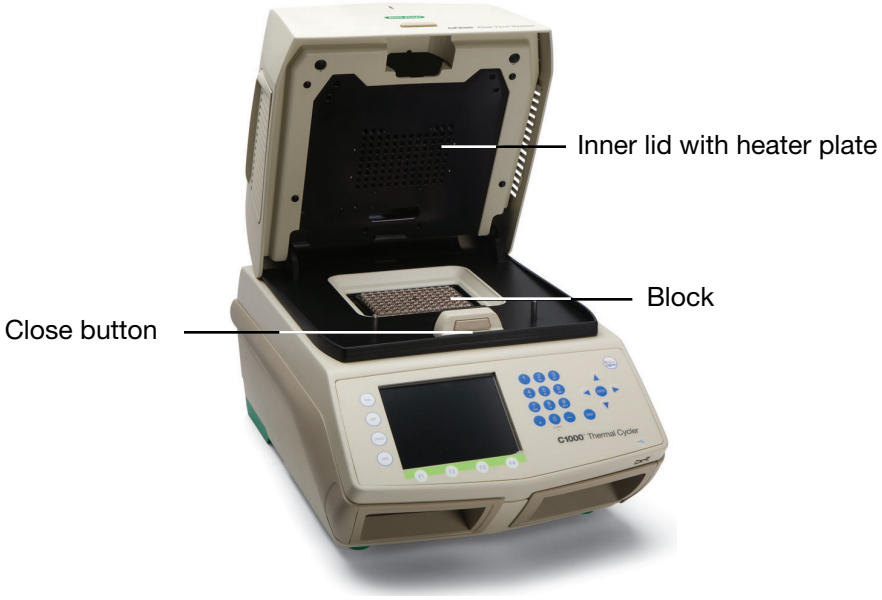


Figure 2. Inside view of the CFX system.

WARNING! Avoid touching the inner lid or block: These surfaces can be hot.

- **Inner lid with heater plate.** The heater lid maintains temperature on the top of the consumable to prevent sample evaporation. Avoid touching or otherwise contaminating the heater plate. Never poke anything through the holes; the optics shuttle system could be damaged
- **Block.** Load samples in this block before the run
- **Close button.** Press this button on the inside of the lid to close the motorized lid

  WARNING! Prevent contamination of the instrument by spills, and never run a reaction with an open or leaking sample lid. For information about general cleaning and maintenance of the instrument, see “Instrument Maintenance” (page 101).

The back panel of the C1000 chassis includes these features (Figure 3):

- **Power switch.** Press the power switch to turn on the power to the system
- **Power input.** Plug in the power cord here
- **Ethernet port.** Connect an ethernet cable to email run logs and stand-alone data files
- **USB connections.** Use these ports to connect the CFX system to a computer or to connect an S1000™ thermal cycler

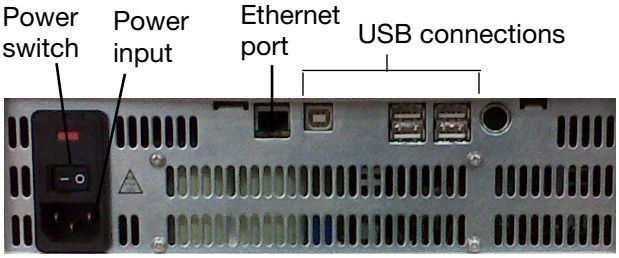


Figure 3. Back panel of C1000 thermal cycler.

WARNING! Do not touch the back of the CFX system when the instrument is on.

Setting Up the System

The CFX system should be installed on a clean, dry, level surface with sufficient cool airflow to run properly. The CFX system can be run in two modes: stand-alone or software controlled. If you are running the system under software-controlled mode, make sure there is sufficient space for a computer during setup.

Position the C1000 thermal cycler base so that the power switch, which is located on the back panel, is easily accessible.

To insert the CFX optical reaction module into the reaction module bay of the C1000 chassis, follow these instructions:

1. Place the C1000 chassis in a suitable location with the locking bar down. Remove any previously installed reaction modules.

2. Lift the optical reaction module using the handle indents above the side air vents (Figure 4).

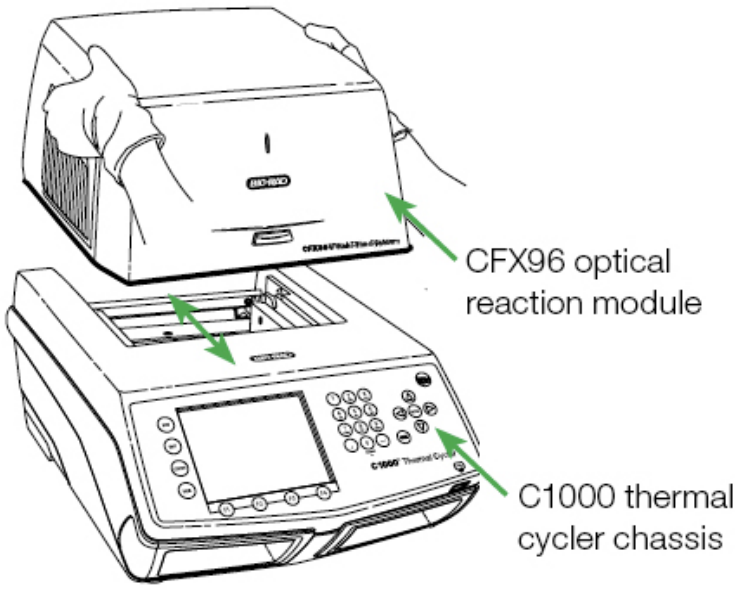


Figure 4. Lifting the optical reaction module into the C1000 chassis.

3. Position the module in the reaction module bay of the C1000 chassis, leaving about 2 cm of space in the front. When in the chassis bay, the optical module should be covering the Bio-Rad logo in front of the bay of the C1000 chassis.

4. Reach around and pull up the locking bar of the C1000 thermal cycler until it is flush with the sides of the module bay. This action moves the module forward, locking it into place (Figure 5)

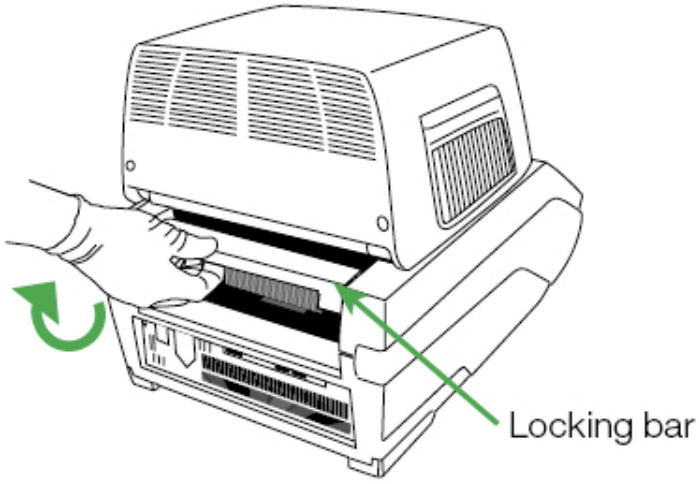


Figure 5. Locking the optical module into place.

5. Check that the module is completely and evenly seated in the C1000 base. There should be no extra space between the module and the base; the space should be even.

6. Plug the power cord into the back of the C1000 base (Figure 3 on page 3) and into an appropriate three-pronged electrical outlet. Press the power switch on the back panel of the C1000 thermal cycler to start the system.

7. Follow the instructions in the front panel of the C1000 thermal cycler to remove the red shipping screw from the inner heater lid (Figure 6). Turn the screw counterclockwise to lift it out of the hole.

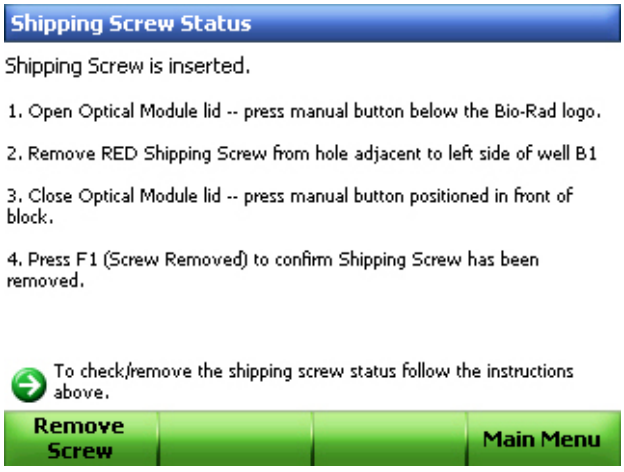


Figure 6. Instructions to remove the shipping screw.

NOTE: If the shipping screw is not removed at this step, it will be detected by CFX Manager software. Follow instructions to remove the screw (page 15).

TIP: The shipping screw must be in place when the module is shipped. Save this screw in a safe place for future shipping.

8. Remove the shipping plate from the thermal cycler block to operate.

Installing CFX Manager Software

CFX Manager software is run on a personal computer (PC) and is required to analyze data from the CFX system and to control the CFX system in software-controlled mode. Table 6 lists computer system requirements for CFX Manager software (version 1.6 or higher).

Table 6. Computer requirements for CFX Manager software version 1.6 or higher

System	Minimum	Recommended
Operating system	Windows 7 and Windows 8	Windows 7 or Windows 8
Drive	CD-ROM drive	CD-RW drive
Hard drive	10 GB	20 GB
Processor speed	2.0 GHz	2.0 GHz
RAM	2 GB RAM	2 GB RAM
Screen resolution	1,024 x 768 with true-color mode	1280 x 1024 with true-color mode
USB	USB 2.0 Hi-Speed port	USB 2.0 Hi-Speed port

To install CFX Manager software:

1. Make sure you are logged in with administrative privileges. The software must be installed on the computer by a user with administrative privileges.

2. Place the CFX Manager software CD in the computer's CD drive.
3. The software launch page should appear automatically. Double-click **Install Software** on the software launch page (Figure 7).

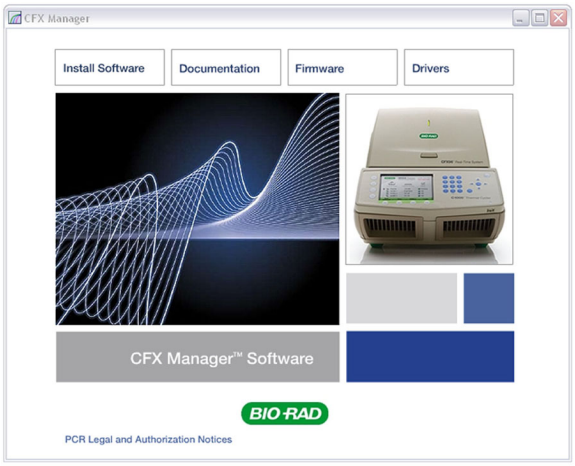


Figure 7. Software installation screen.

4. Follow the instructions on screen to complete installation. When completed, the Bio-Rad CFX manager software icon will appear on the desktop of the computer.
5. If the launch page does not appear automatically, double-click on (**CD drive):\Bio-Rad CFX**, then open and follow instructions in the Readme.txt file. See "Installing the Software Manually" on page 103.

Installing the Drivers

If the CFX system is going to be run in **Software-controlled mode**, drivers must be installed on the computer. Use only the supplied USB cable, which is sufficiently shielded to prevent data loss.

To install the system drivers:

1. Connect the C1000 chassis to the computer by plugging a USB cable into the USB 2.0 A port located on the back of the chassis (Figure 3 on page 3), and then connecting the cable into the USB 2.0 B port located on the computer.
2. If it is not already turned on, turn on the system using the power switch on the back of the C1000 chassis. Follow the instructions in the **Found New Hardware Wizard** that launches after the instrument is first detected by the computer.
3. On the first screen, select **Yes, this time only** to instruct the Windows operating system to connect to Windows Update to search for software. Click **Next**.
4. Instruct the wizard to **Install the software automatically**. Click **Next** to continue installing the drivers.
5. Click **Finish** at the software installation completion screen when the drivers are installed.

Software Files

CFX Manager software stores information about experiments in specific files (Table 7):

Table 7. Open these file types with CFX Manager software.

File Type	Extension	How to View and Edit File
Protocol	.prcl	Select in Experiment Setup and edit in Protocol Editor
Plate	.pltd	Select in Experiment Setup and edit in Plate Editor
Data	.pcrd	View and analyze in Data Analysis window
LIMS	.plrn	Contains plate setup and protocol information required to conduct a LIMS compatible run
Gene Study	.mgxd	View and analyze in Gene Study window
Stand-alone pre-data file	.zpcr	Contains fluorescence readings from stand-alone operation that is converted into a data file

Running Experiments

Recommended Plastic Consumables

The CFX system accepts low profile 0.2 ml plates and tubes. Bio-Rad recommends the following consumables for optimal results:

- **MLL-9601.** Low-profile 96-well unskirted plates with clear wells
- **MLL-9651.** Low-profile 96-well unskirted plates with white wells
- **HSP-9601.** Hard-Shell 96-well skirted plates with white shell and clear wells
- **HSP-9655.** Hard-Shell 96-well skirted plates with white shell and white wells
- **TLS-0801.** Low-profile 0.2 ml 8-tube strips without caps, clear wells
- **TLS-0851.** Low-profile 0.2 ml 8-tube strips without caps, white wells
- **TCS-0803.** Optical flat 8-cap strips, for 0.2 ml tubes and plates

NOTE: High profile plates can also be used with the CFX96 Deep Well system.

Plate Seals:

- **MSB-1001.** Microseal® 'B' adhesive seals, optically clear (strong adhesive-based)
- **MSC-1001.** Microseal 'C' optical seals, optically clear (pressure-activated adhesivebased)

Loading the Block

To load your reactions in the block, follow these suggestions:

- Click the **Open Lid** button located on software's Start Run tab (see "Start Run Tab" on page 19), or press the lid button on the front of the system (Figure 1) to start opening the motorized lid.
- Place the 0.2 ml microplate or tube strips with sealed lids in the block. Check that the tubes are completely sealed to prevent leakage. For optimal results, load sample volumes of 10–25 µl for the CFX96 system and 10–125 µl for the CFX96 Deep Well system.

  NOTE: For accurate data analysis, check that the orientation of reactions in the block is exactly the same as the orientation of the well contents in the software Plate tab. If needed, edit the well contents before, during, or after the run.

WARNING! When running the CFX system, always balance the tube strips or cut microplates in the wells (Figure 8). For example, if you run one tube strip on the left side of the block, run an empty tube strip (with caps) on the right side of the block to balance the pressure applied by the heated lid. Failure to balance the pressure can result in sample evaporation and failed runs.

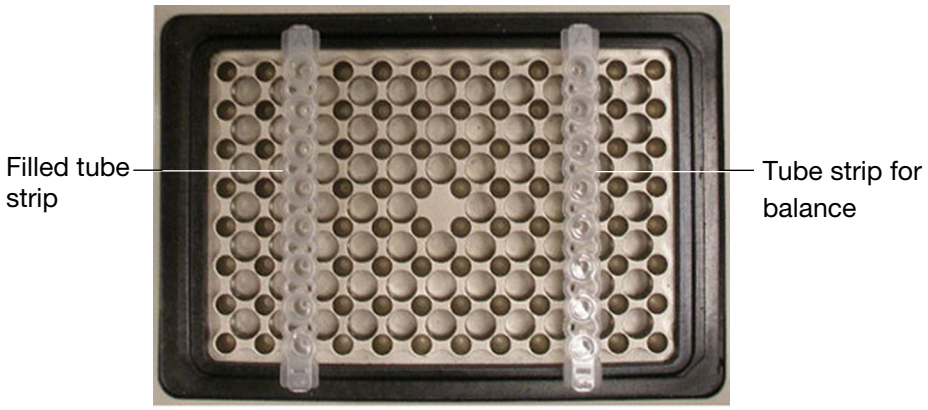


Figure 8. Balance the tube strips or cut microplates in the block.

WARNING! Be sure that nothing is blocking the lid when it closes. Although there is a safety mechanism to prevent the lid from closing if it senses an obstruction, do not place anything in the way of the closing lid.

Shutting Down the System

To shut down the CFX system, follow these suggestions:

- After a run, click the open lid button on the front of the CFX system to access the samples loaded in the thermal cycler block.
- Remove the samples from the block and click the close lid button to close the lid of the CFX system.
- Press the power switch on the back panel of the C1000 thermal cycler to power down the system.

2 CFX Manager™ Software

Read this chapter for information about getting started with CFX Manager software.

- Main software window (page 9)
- Startup Wizard (page 12)
- Detected Instruments pane (page 13)
- Instrument Properties window (page 14)

Main Software Window

Features available in the main software window are provided in Figure 9.

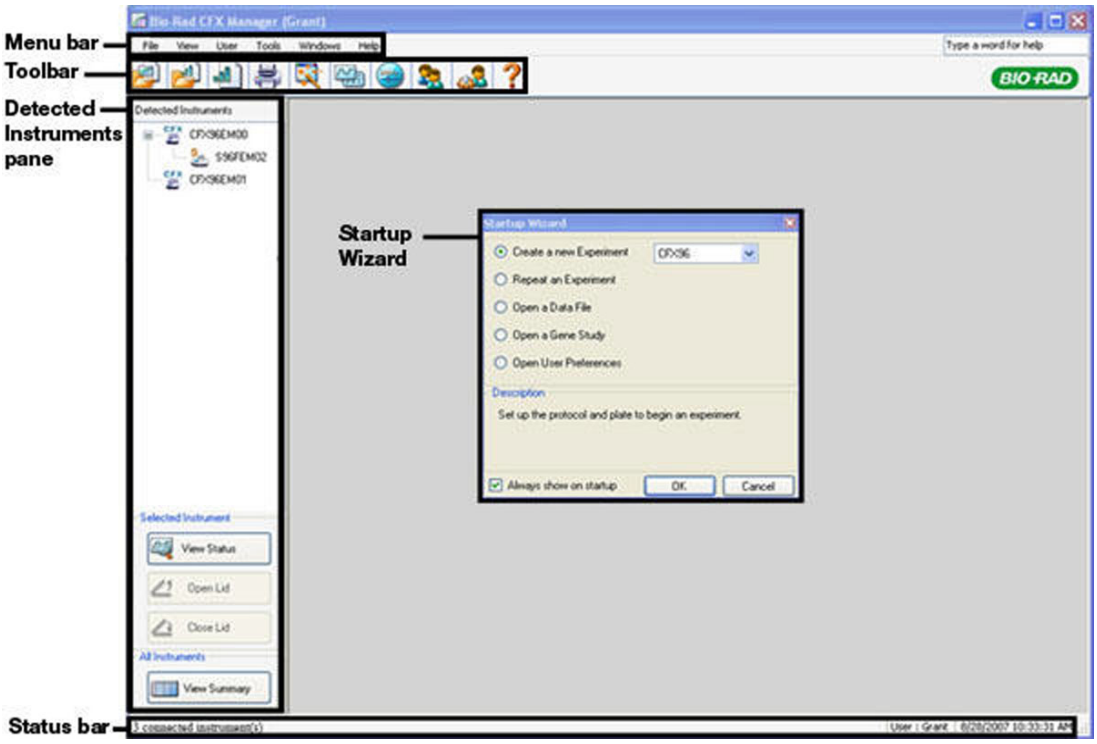


Figure 9. The main software window.

Menu Bar

The menu bar of the main software window provides the items listed in Table 8.

Table 8. Menu bar items in the main software window.

Menu Item	Command	Function
File	New	Create a new protocol, plate, experiment, or Gene Study
	Open	Open existing files, including protocol (.prcl), plate (.pltd), data (.pcrd), and Gene Study (.mgxd) files, LIMS (.plrn), stand-alone run files (.zpcr)
	Recent Data Files	View a list of the ten most recently viewed data files, and select one to open in Data Analysis
	Repeat an Experiment	Open the Experiment Setup window with the protocol and plate from a completed run to quickly repeat the run
	Exit	Exit the software program
View	Application Log	Display the application log for the software
	Run Reports	Select a run report to review from a list
	Startup Wizard	Open the Startup Wizard
	Experiment Setup	Open the Experiment Setup window
	Instrument Summary	Open the Instrument Summary window
	Detected Instruments	Show or hide the Detected Instruments pane
	Toolbar	Show or hide the main software window toolbar
	Status Bar	Show or hide the main software window status bar
User	Select User	Open the Select User window to change software users
	Change Password	Change your user password
	User Preferences	Open the User Preferences window
	User Administration	Manage users in the User Administration window
Tools	Dye Calibration Wizard	Open the Dye Calibration window to calibrate an instrument for a new fluorophore
	Protocol AutoWriter	Open the Protocol AutoWriter window to create a new protocol
	Ta Calculator	Open the Ta Calculator window to calculate the annealing temperature of primers
	View Block Status Log	View a log of the thermal cycler block
	Application Data Folder	Open the Application Data folder to view software files
	User Data Folder	Open the Data folder to view protocol, plate, and data files
	Properties All Instruments	View properties of all detected instruments, including serial numbers
	Zip Data and Log Files	Choose and condense selected files in a zipped file for storage or to email
	Options	Configure software email

Table 8. Menu bar items in the main software window.

Menu Item	Command	Function
Windows	Cascade	Arrange software windows on top of each other
	Tile Vertical	Arrange software windows from top to bottom
	Tile Horizontal	Arrange software windows from right to left
	Close All	Close all open software windows
Help	Contents	Open the software Help for more information about running PCR and real-time PCR
	Index	View the index in the software Help
	Search	Search the software Help
	Gene Expression Gateway Website	Open a website to find information about running PCR and real-time PCR experiments
	PCR Reagents Website	View a website that lists Bio-Rad consumables for PCR and real-time PCR reagents
	PCR Plastic Consumables Website	View a website that lists Bio-Rad consumables for PCR and real-time PCR experiments
	Software Updates	Check for software updates from Bio-Rad
	About	Open a window to see the software version

Toolbar Buttons

Click a button in the toolbar of the main software window (Table 9) for quick access to common software commands.

Table 9. Toolbar buttons in the main software window.

Button	Button Name	Function
	Open a Data File	Open a browser window to locate a data file (*.pcrd extension) and open it in the Data Analysis window (page 53)
	Open a Gene Study	Open a browser window to locate a Gene Study file (.mgxd extension) and open it in the Gene Study window (page 87)
	Create a New Gene Study	Open the Gene Study window (page 87) to add files and create a new study
	Print	Print the current software window
	Startup Wizard	Open the Startup Wizard that links you to common software functions (page 12)

Table 9. Toolbar buttons in the main software window.

Button	Button Name	Function
	Experiment Setup	Open the Experiment Setup window to run an experiment (page 17)
	Protocol AutoWriter	Open the Protocol AutoWriter window to create a new protocol (page 30)
	Select User	Open the Select User window to change software users (see “Log In or Select User” on page 91)
	User Preferences	Open the User Preferences window (page 92)
	Help	Open the software Help window for more information about running PCR and real-time PCR

Startup Wizard

The Startup Wizard automatically appears when CFX Manager software is first opened (Figure 9). If it is not shown, click the **Startup Wizard** button on the main software window toolbar.

Options in the Startup Wizard include the following:

- **Create a new Experiment (page 17).** Set up the protocol and plate to begin a new experiment.
- **Repeat an Experiment.** Set up an experiment with the protocol and plate from a completed run. If needed, you can edit the experiment before the run
- **Open a Data File (page 53).** Open a data file to analyze results
- **Open a Gene Study (page 86).** Open a multifile gene expression study to analyze results from multiple gene expression experiments
- **Open User Preferences (page 92).** Open the User Preferences window to customize software settings

Detected Instruments Pane

Connected instruments appear in the **Detected Instruments** pane (Figure 10). This list shows each instrument as an icon named with the serial number (default).

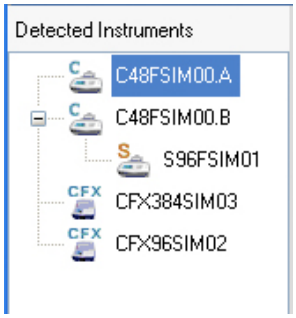


Figure 10. Instruments listed in the Detected Instruments pane.

Right-click on the instrument icon or block to select one of these options:

- **View Status.** Open the Run Details window to check the status of the selected instrument block
- **Flash Block Indicator.** Flash the indicator LED on the instrument
- **Open Lid.** Open a motorized lid on the selected instrument block
- **Close Lid.** Close a motorized lid on the selected instrument block
- **Rename.** Change the name of the instrument
- **Properties.** Open the Instrument Properties window
- **Collapse All.** Collapse the list of instruments in the Detected Instruments pane
- **Expand All.** Expand the list of instruments in the Detected Instruments pane

You can also control a block by clicking an instrument block icon in the Detected Instrument pane and then clicking a button in the Selected Instrument pane (Figure 11).

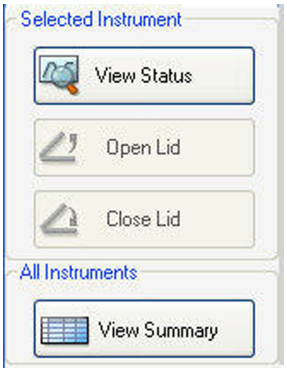


Figure 11. Buttons at the bottom of the Detected Instruments pane.

- Click **View Status** to open the Run Details window to check the status of the selected instrument block
- Click **Open Lid** to open the motorized lids on the selected instrument
- Click **Close Lid** to close the motorized lids on the selected instrument
- Click **View Summary** to open the Instrument Summary window

If only one instrument is detected, then the **View Summary** button does not appear. To view the Instrument Summary window for a single instrument, select **View > Instrument Summary**.

Status Bar

The left side of the status bar at the bottom of the main software window shows the current status of instruments. View the right side of the status bar to see the current user name, date, and time.

Click and drag the right corner of the status bar to resize the main window.

Instrument Properties Window

To open the Instrument Properties window to view information about an instrument, right-click on the instrument icon in the Detected Instruments pane (Figure 10 on page 13).

Properties Tab

The Properties tab displays important serial numbers for the connected instrument, including the thermal cycler and reaction module. The firmware versions are also displayed. The default name for an instrument is the C1000 thermal cycler serial number, which appears in many locations, including the Detected Instruments pane (Figure 12).

To rename an instrument for ease of identification, follow these instructions:

- In the Instrument Properties tab, type a name in the **Rename** box at the top of the Properties tab and hit the **Rename** button to save the new name

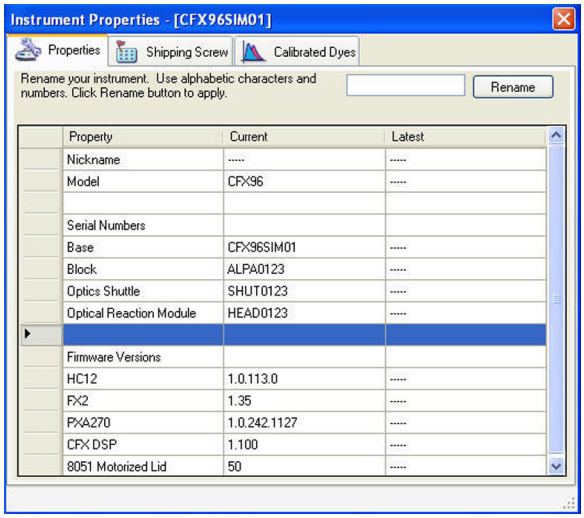


Figure 12. Instrument Properties window.

Shipping Screw Tab

The Shipping Screw tab includes instructions for installing or removing the red shipping screw. To prevent damage to the optical reaction modules, install the shipping screw any time you ship the CFX system.

> NOTE: If the shipping screw is detected by the software, the Instrument Properties window automatically opens with the Shipping Screw tab in front. Follow the instructions to remove the screw. You can not perform a run with the shipping screw installed.

The information in this tab changes depending on whether the shipping screw is installed or removed. For example, to install the shipping screw, click the **Install Shipping Screw** button and follow the instructions in the tab (Figure 13).

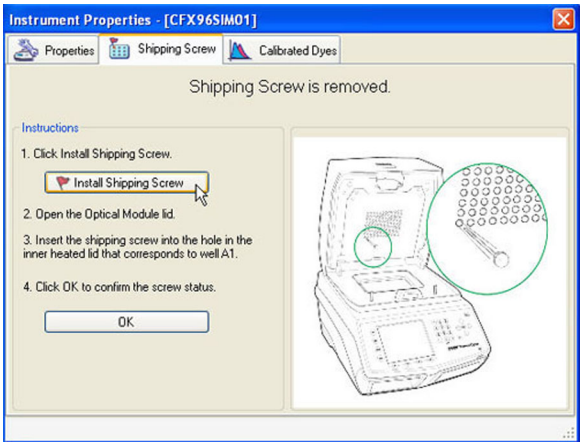
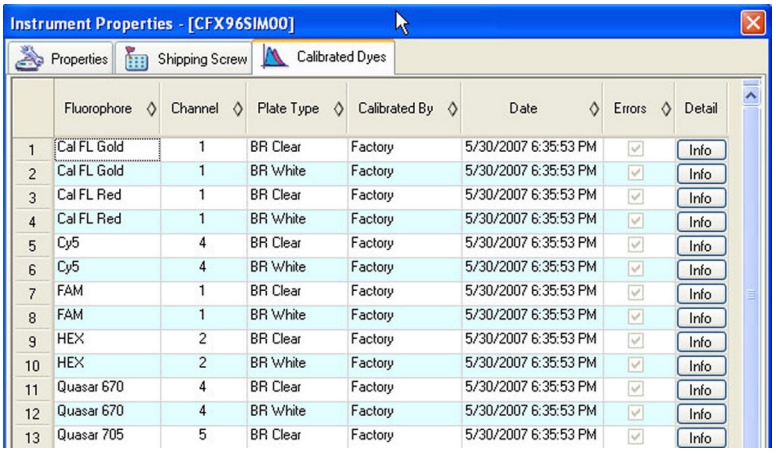


Figure 13. Instructions for installing the shipping screw.

Calibrated Dyes Tab

Open the Calibrated Dyes tab to view the list of calibrated fluorophores and plates for the selected instrument (Figure 14). Click an **Info** button to see detailed information about a calibration.



	Fluorophore	Channel	Plate Type	Calibrated By	Date	Errors	Detail
1	Cal FL Gold	1	BR Clear	Factory	5/30/2007 6:35:53 PM	☑	Info
2	Cal FL Gold	1	BR White	Factory	5/30/2007 6:35:53 PM	☑	Info
3	Cal FL Red	1	BR Clear	Factory	5/30/2007 6:35:53 PM	☑	Info
4	Cal FL Red	1	BR White	Factory	5/30/2007 6:35:53 PM	☑	Info
5	Cy5	4	BR Clear	Factory	5/30/2007 6:35:53 PM	☑	Info
6	Cy5	4	BR White	Factory	5/30/2007 6:35:53 PM	☑	Info
7	FAM	1	BR Clear	Factory	5/30/2007 6:35:53 PM	☑	Info
8	FAM	1	BR White	Factory	5/30/2007 6:35:53 PM	☑	Info
9	HEX	2	BR Clear	Factory	5/30/2007 6:35:53 PM	☑	Info
10	HEX	2	BR White	Factory	5/30/2007 6:35:53 PM	☑	Info
11	Quasar 670	4	BR Clear	Factory	5/30/2007 6:35:53 PM	☑	Info
12	Quasar 670	4	BR White	Factory	5/30/2007 6:35:53 PM	☑	Info
13	Quasar 705	5	BR Clear	Factory	5/30/2007 6:35:53 PM	☑	Info

Figure 14. Calibrated Dyes tab in the Instrument Properties window.

3 Running Experiments

Read this chapter for information about running experiments using CFX Manager™ software:

- Experiment Setup window (page 17)
- Protocol tab (page 18)
- Plate tab (page 19)
- Start Run tab (page 19)
- Run Details window (page 20)

Experiment Setup Window

The Experiment Setup window provides quick access to the files and settings needed to set up and run an experiment. To open the Experiment Setup window, follow one of these options:

- Click **Create a New Experiment** option in the Startup Wizard (page 12)
- Click the **Experiment Setup** button in the main software toolbar (page 17)
- Select **File > New > Experiment** in the main software menu bar (page 10)

The Experiment Setup window includes three tabs:

- **Protocol.** Click the Protocol tab to select an existing protocol to run or edit, or to create a new protocol in the Protocol Editor window (page 25)
- **Plate.** Click the Plate tab to select an existing plate to run or edit, or to create a new plate in the Plate Editor window (page 33)
- **Start Run.** Click the Start Run tab (page 19) to check the run settings, select one or more instrument blocks, and begin the run

  NOTE: If the protocol currently selected in the Protocol tab does not include a step with a plate read for real-time PCR analysis, then the Plate tab is hidden. To view the Plate tab, add a “Plate Read” (page 27) in at least one step in the protocol.

  NOTE: Start a new experiment from a previous run by selecting **File > Repeat an Experiment** in the main software menu bar. Then select the data file (.pcrd) for the experiment you want to repeat.

The Experiment Setup window opens with the Protocol tab in front (Figure 15). To open another tab, click that tab or click **Prev** and **Next** buttons at the bottom of the window.

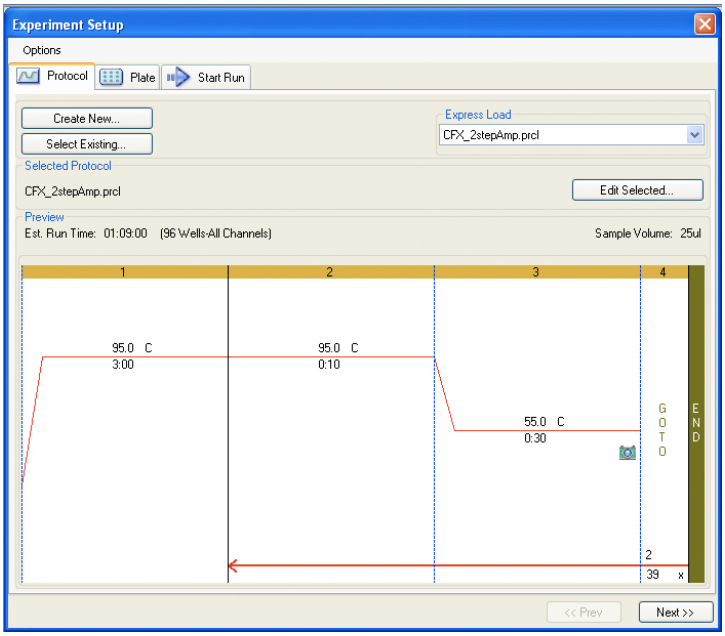


Figure 15. Experiment Setup window, including the Protocol, Plate, and Start Run tabs.

Protocol Tab

The Protocol tab shows a preview of the selected protocol file loaded in the Experiment Setup (Figure 15). A protocol file contains the instructions for the instrument temperature steps and instrument options that control the ramp rate and lid temperature.

Select one of the following options to select an existing protocol, create a new protocol, or edit the currently selected protocol:

- **Create New button.** Open the Protocol Editor to create a new protocol
- **Select Existing button.** Open a browser window to select and load an existing protocol file (.prcl extension) into the Protocol tab
- **Express Load pull-down menu.** Quickly select a protocol to load it into the Protocol tab

  TIP: To add or delete protocols in the **Express Load** menu, add or delete files (.prcl extension) in the **Express Load** folder. To locate this folder, select **Tools > User Data Folder** in the menu bar of the main software window
- **Edit Selected button.** Open the currently selected protocol in the Protocol Editor

End Point Only Runs

To run a protocol that contains only an end point data acquisition step, select **Options > End Point Only Run** from Options in the menu bar of the Experiment Setup window. The default end point protocol, which includes two cycles of 60.0°C for 30 seconds, is loaded into the Protocol tab. To change the step temperature or sample volume for the end point only run, click the **Start Run** tab and edit the **Step Temperature** or **Sample Volume**.

Plate Tab

The Plate tab shows a preview of the selected plate file loaded in the Experiment Setup (Figure 16). In a real-time PCR experiment, the plate file contains a description of the contents of each well, the scan mode, and the plate type. CFX Manager software uses these descriptions for data collection and analysis.

Select one of the following options to select an existing plate, create a new plate, or edit the currently selected plate:

- **Create New button.** Open the Plate Editor to create a new plate
- **Select Existing button.** Open a browser window to select and load an existing plate file (.pltd extension) into the Plate tab
- **Express Load pull-down menu.** Quickly select a plate to load it into the Plate tab

  TIP: To add or delete plates in the **Express Load** menu, add or delete files (.pltd extension) in the **Express Load** folder. To locate this folder, select **Tools > User Data Folder** in the menu bar of the main software window.
- **Edit Selected button.** Open the currently selected plate in the Plate Editor

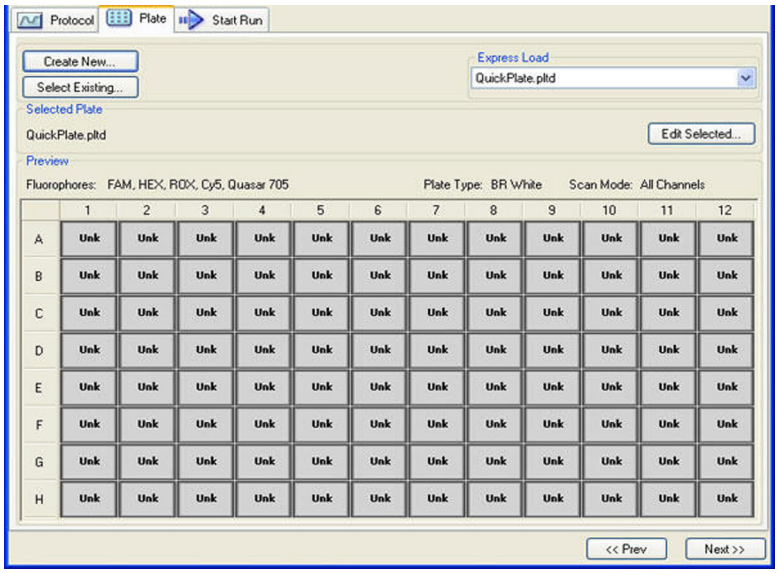


Figure 16. Plate tab window.

Start Run Tab

The Start Run tab (Figure 17) includes a section for checking information about the run that is going to be started, including the selected protocol and plate files, and a section for selecting the instrument block.

- **Run Information pane.** View the selected Protocol file, Plate file, and data acquisition Scan Mode setting. Enter optional notes about the experiment in the **Notes** box

- **Start Run on Selected Block(s)** pane. Select one or more blocks, edit run parameters (if necessary), and then click the **Start Run** button to begin the experiment

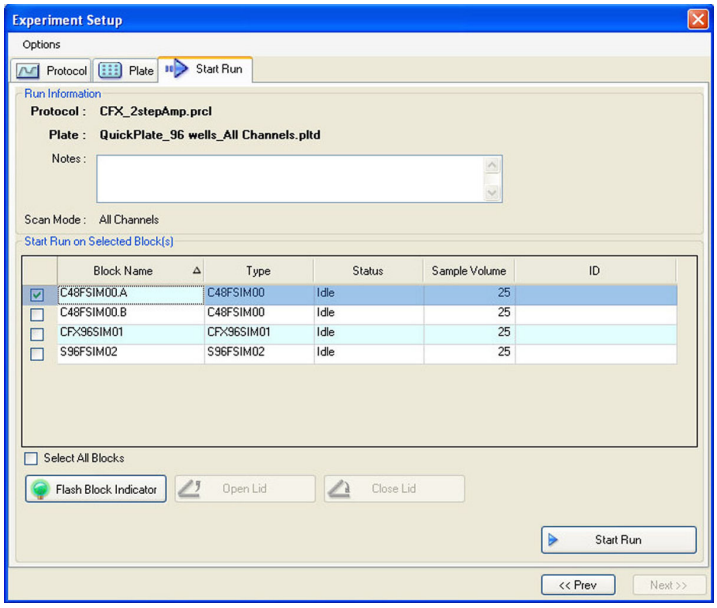


Figure 17. The Start Run tab.

To add or remove run parameters from the spreadsheet in the **Start Run on Selected Block(s)** pane, right-click on the list and select an option in the menu to display. Choose the value to change by clicking the text inside the cell to select it and then typing in the cell or by selecting a new parameter from the pull-down menu. Editable parameters include:

- **Lid Temperature.** View the temperature of the lid. Override the default lid temperature by selecting the text and typing a new temperature

  WARNING! Changing the lid temperature can impact experiment results and may result in failed runs.

Buttons for Controlling the Instrument

Click the following buttons in the Start Run tab to remotely operate the selected instruments:

- **Start Run.** Start the experiment on the selected instrument blocks
- **Flash Block Indicator.** Flash the indicator LED on the selected instrument blocks
- **Open Lid.** Open motorized lid on selected instrument blocks
- **Close Lid.** Close motorized lid on selected instrument blocks

Run Details Window

When you click the **Start Run** button, CFX Manager software prompts you to save the name of the data file and then opens the Run Details window. Review the information in this window to monitor the progress of a run.

- **Run Status tab.** Check the current status of the protocol, open the lid, pause a run, add repeats, skip steps, or stop the run
- **Real-Time Status tab.** View the real-time PCR fluorescence data as they are collected
- **Time Status tab.** View a full-screen countdown timer for the protocol

Figure 18 shows the features of the Run Details window.

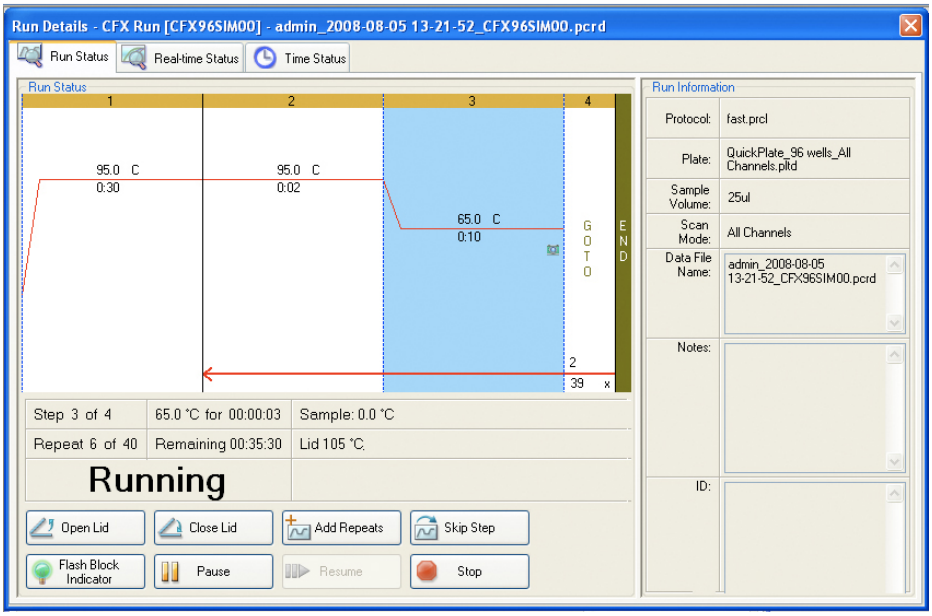


Figure 18. Run Details window.

Run Status Tab

The Run Status tab (Figure 18) shows the current status of a run in progress in the Run Details window and provides buttons (page 21) to control the lid and change the run in progress.

- **Run Status pane.** Displays the current progress of the protocol, including the current step, current GOTO repeat, block temperature, remaining hold time for the current step, sample temperature, lid and shuttle temperature
- **Run Status buttons.** Click one of the buttons to remotely operate the instrument or to interrupt the current protocol
- **Run Information pane.** Displays experiment details

Run Status Tab Buttons

Click one of the buttons listed in Table 10 to operate the instrument from the software, or to change the run that is in progress.

NOTE: Changing the protocol during the run, such as adding repeats, does not change the protocol file associated with the run. These actions are recorded in the Run Log.

Table 10. Run Status buttons and their functions

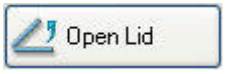
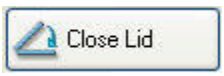
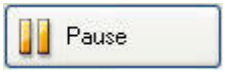
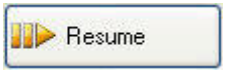
Button	Function
	Open the motorized lid on selected instruments **WARNING!** Opening the lid during a run pauses the run during the current step and might alter the data.
	Close the motorized lid on selected instruments

Table 10. Run Status buttons and their functions (continued)

Button	Function
	Add more repeats to the current GOTO step in the protocol. This button is only available when a GOTO step is running.
	Skip the current step in the protocol. If you skip a GOTO step, the software verifies that you want to skip the entire GOTO loop and proceed to the next step in the protocol.
	Flash the LED on the selected instrument to identify the selected blocks
	Pause the protocol NOTE: This action is recorded in the Run Log.
	Resume a protocol that was paused
	Stop the run before the protocol ends **WARNING!** Stopping a run prematurely may alter your data and may result in a failed experiment.

Real-Time Status Tab

The **Real-time Status** tab (Figure 19) shows real-time PCR data collected at each cycle during the protocol after the first two plate reads. This tab also shows the well selector and text describing the protocol status at the bottom of the window.

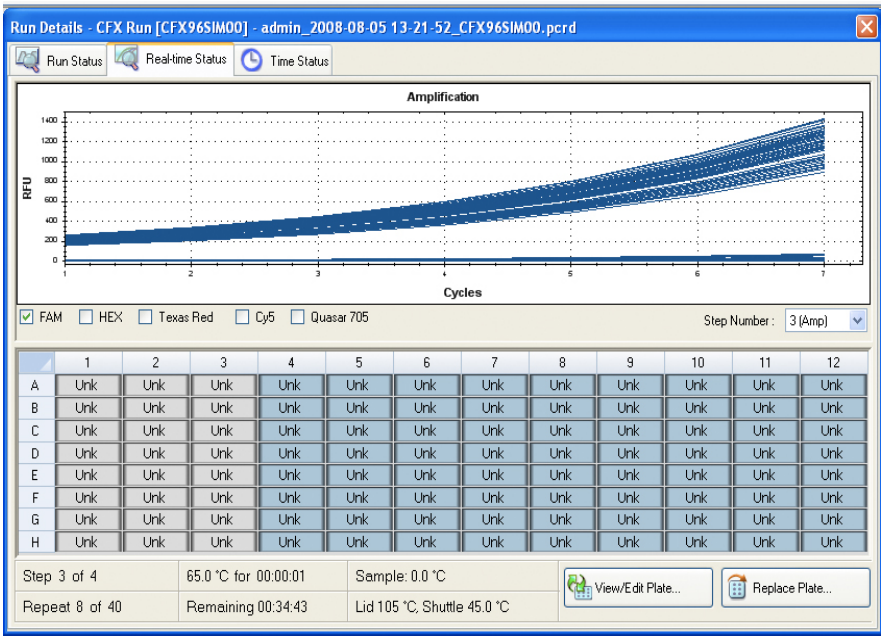


Figure 19. The Real-time Status tab displays the data during a run.

Replacing a Plate File

During a run, replace the plate file by clicking the **Replace Plate** button (Figure 19) in the **Real-time Status** tab. Select the new plate file (.pltd) from the list in the windows browser.

NOTE: CFX Manager software checks the scan mode and plate size for the plate file; these must match the run settings that were started during the experiment.

TIP: Replacing a plate file is especially useful if you start a run with a Quick Plate file in the Express Load folder.

Time Status Tab

The **Time Status** tab shows a countdown timer for the current run.

4 Protocols

Read the following chapter for information about creating and editing protocol files:

- Protocol Editor window (page 25)
- Protocol Editor controls (page 27)
- Temperature control mode (page 30)
- Protocol AutoWriter (page 30)

Protocol Editor Window

A protocol instructs the instrument to control the temperature steps, lid temperature, and other instrument options. Open the Protocol Editor window to create a new protocol or to edit the protocol currently selected in the Protocol tab. Once a Protocol is created or edited in the Protocol Editor, click **OK** to load the protocol file into the Experiment Setup window and run it.

WARNING! Always confirm the loaded protocol is correct in the Start Run tab before beginning the run. Performing a run with the wrong protocol could alter the data or result in a failed run.

Opening the Protocol Editor

To open the Protocol Editor, follow one of these options:

- To create a new protocol, select **File > New > Protocol** or click the **Create New** button in the Protocol tab (page 18)
- To open an existing protocol, select **File > Open > Protocol**, or click the **Open Existing** button in the Protocol tab (page 18)
- To edit the current protocol in the Protocol tab, click the **Edit Selected** button in the Protocol tab (page 18)

  TIP: To change the default settings in the Protocol Editor window, enter the changes in the **Protocol** tab in the **User Preferences** window (page 92)

Protocol Editor Window

The Protocol Editor window (Figure 20) includes the following features:

- **Menu bar.** Select settings for the protocol
- **Toolbar.** Select options for editing the protocol

- **Protocol.** View the selected protocol in a graphic (top) and text (bottom) view. Click the temperature or dwell time in the graphic or text view of any step to enter a new value
- **Protocol Editor buttons.** Edit the protocol by clicking one of the buttons to the left of the text view

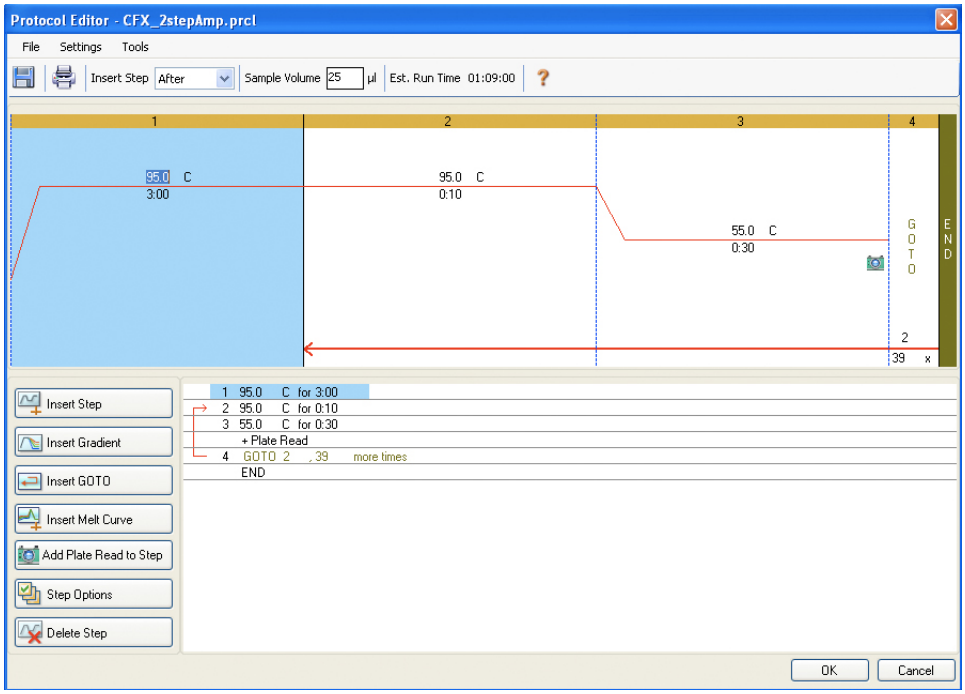


Figure 20. Protocol Editor window with buttons for editing protocols.

Protocol Editor Menu Bar

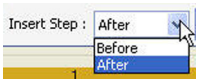
The menu bar in the Protocol Editor window provides the menu items listed in Table 11

Table 11. Protocol Editor menu bar

Menu Item	Command	Function
File	Save	Save the current protocol
	Save As	Save the current protocol with a new name or in a new location
	Close	Close the Protocol Editor
Settings	Lid Settings	Open the Lid Settings window to change or to set the Lid Temperature
Tools	Gradient Calculator	Select the block type for a gradient step.
	Run-time Calculator	Select the instrument and scan mode to be used for calculating the estimated run time in the Experiment Setup window

The toolbar in the Protocol Editor window provides quick access for important functions. Table 12 lists the function of the Protocol Editor toolbar buttons.

Table 12. Protocol Editor toolbar buttons

Toolbar Button and Menus	Name	Function
	Save	Save the current protocol file
	Print	Print the selected window
	Insert Step	Select **After** or **Before** to insert a step relative to the currently highlighted step
	Sample Volume	Enter a sample volume in µl between 0 and 50. Sample volume determines the Temperature Control mode (page 30). Enter zero (0) to select Block mode
	Run Time	View an estimated run time based on the protocol steps and ramp rate
	Help	Open the software Help for more information about protocols

Protocol Editor Controls

The Protocol Editor window includes buttons for editing the protocol on the bottom left of the screen. First, select and highlight a step in the protocol by left clicking it with the mouse pointer. Then click one of the Protocol Editor buttons at the bottom left side of the Protocol Editor window. The location for inserting a new step **Before** or **After** the currently selected step is determined by the status of the **Insert Step** box located in the toolbar.

Insert Step Button

To insert a temperature step before or after the currently selected step:

1. Click the **Insert Step** button.
2. Edit the temperature or hold time by clicking the default value in the graphic or text view, and entering a new value.

Add or Remove a Plate Read

To add a plate read to a step or to remove a plate read from a step:

1. Select the step by clicking the step in either the graphical or text view.
2. Click the **Add Plate Read to Step** button to add a plate read to the selected step. If the step already contains a plate read, the text on the button changes, so now the same button reads **Remove Plate Read**. Click to remove a plate read from the selected step.

Insert Gradient Button

To insert a gradient step before or after the currently selected step:

1. Insert a temperature gradient step by clicking the **Insert Gradient** button.
2. Edit the gradient temperature range by clicking the default temperature in the graphic or text view, and entering a new temperature.
3. Edit the hold time by clicking the default time in the graphic or text view, and entering a new time.

Figure 21 shows the inserted gradient step. The temperatures of each row in the gradient are charted on the right side of the window.

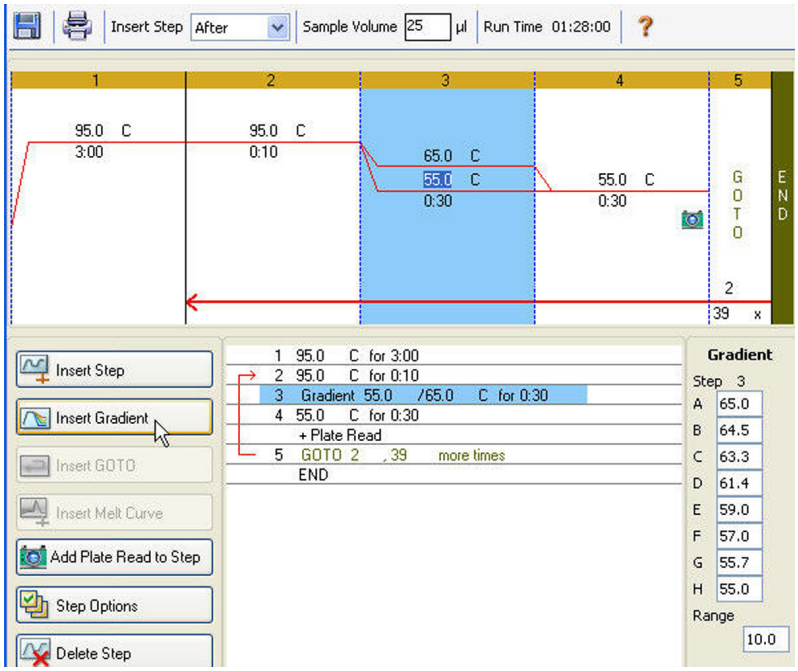


Figure 21. Protocol with inserted gradient step.

Insert GOTO Button

To insert a GOTO step before or after the selected step:

1. Click the **Insert GOTO** button.
2. Edit the GOTO step number or number of GOTO repeats by clicking the default number in the graphic or text view and entering a new value.

Figure 21 shows an inserted GOTO step at the end of the protocol. Notice that the GOTO loop includes steps 2 through 4.

Insert Melt Curve Button

To insert a melt curve step before or after the selected step:

1. Click the **Insert Melt Curve** button.

2. Edit the melt temperature range or increment time by clicking the default number in the graphic or text view, and entering a new value. Alternatively, click the **Step Options** button to enter the gradient range in the Step Options window (page 29).

   NOTE: You cannot insert a melt curve step inside a GOTO loop.

   NOTE: The melt curve step includes a 30 second hold at the beginning of the step that is not shown in the protocol.

Figure 22 shows a melt curve step added after step 6.

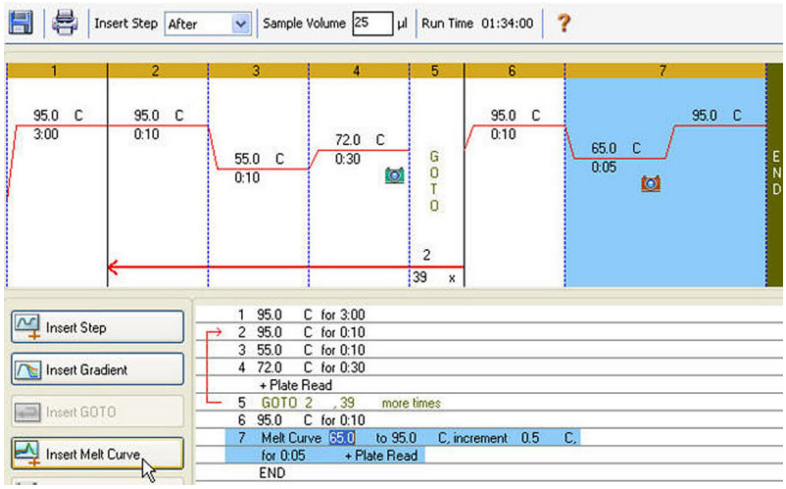


Figure 22. Protocol with inserted melt curve step.

Step Options

To change a step option for the selected step:

1. Select a step by clicking on the step in the graphic or text view.
2. Click the **Step Options** button to open the Step Options window.
3. Add or remove options by entering a number, editing a number, or clicking a check box.

   TIP: To hold a step forever (an infinite hold), enter zero (0.00) for the time.

Figure 23 shows the selected step with a gradient of 10°C. Notice that some options are not available in a gradient step. A gradient step cannot include an increment or ramp rate change.

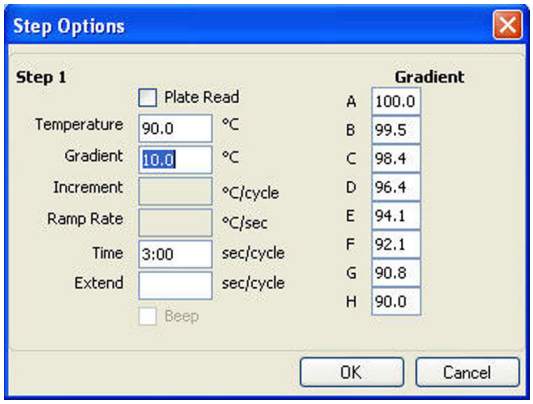


Figure 23. Step option for a gradient.

The **Step Options** window lists the following options you can add or remove from steps:

- **Plate Read**. Check the box to include a plate read
- **Temperature**. Enter a target temperature for the selected step
- **Gradient.** Enter a gradient range for the step
- **Increment.** Enter a temperature to increment the selected step; the increment amount is added to the target temperature with each cycle
- **Ramp Rate.** Enter a rate for the selected step; the range depends on the block size
- **Time.** Enter a hold time for the selected step
- **Extend.** Enter a time to extend the selected step. The extend amount is added to the hold time with each cycle
- **Beep.** Check the box to include a beep at the end of the step

  TIP: When you enter a number that is outside the option range, the software changes the number to the closest entry within the range.

Delete Step Button

To delete a step in the protocol:

1. Select a step in the graphic or text view.
2. Click the **Delete Step** button to delete the selected step.

   WARNING! You cannot undo this function.

Temperature Control Mode

The instrument uses one of two temperature control modes to determine when the sample reaches the target temperature in a protocol.

Enter a sample volume in the protocol editor to select a temperature control mode:

- **Calculated mode.** When you enter a sample volume between 1 and 50 µl, the thermal cycler calculates the sample temperature based on volume. This is the standard mode
- **Block mode.** When you enter a sample volume of zero (0) µl, the thermal cycler records the sample temperature as the same as the measured block temperature

Protocol AutoWriter

Open the Protocol AutoWriter to quickly write protocols for PCR and real-time PCR experiments. To open the Protocol AutoWriter, select one of these options:

- Click the **Protocol AutoWriter** button in the main software window toolbar
- Select **Tools > Protocol AutoWriter** from the menu bar in the main software window

Figure 24 shows a protocol (bottom of the window) written by the Protocol AutoWriter.

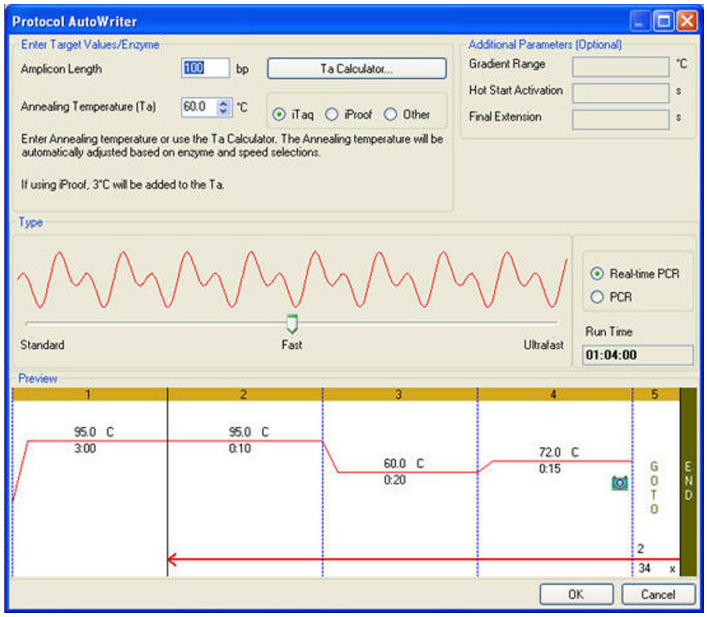


Figure 24. Protocol AutoWriter window with a new protocol.

Creating a Protocol With the Protocol AutoWriter

The Protocol AutoWriter window uses information about your reaction to automatically generate a protocol file. Follow these steps to use the Protocol AutoWriter to create a new protocol:

1. Click the **Protocol AutoWriter** button on the toolbar to open the Protocol AutoWriter window.
2. Enter the **Annealing Temperature (Ta)** and **Amplicon Length** in the boxes within the Enter Target Values/Enzymes pane. If you do not know the annealing temperature for primers, click the **Ta Calculator** button to enter the primer sequences and calculate the annealing temperature. For information about the calculations used in the Ta Calculator see Breslauer et al. 1986.
3. Select an enzyme type from the list of options (**iTaq, iProof, or Other**).
4. Add parameters in the **Additional Parameters (Optional)** pane if you want to add a Gradient Range, Hot Start Activation temperature, or Final Extension time in the protocol.
5. Select a protocol speed (Standard, Fast, or Ultrafast) by moving the sliding bar in the Type pane. When you move the sliding bar, the software adjusts the total run time. Select **Real-time PCR** to tell the software to collect fluorescence data.
6. Review the protocol in the Preview pane and total run time. Make changes as needed.
7. Click **OK** to save the new protocol, or click **Cancel** to close the window without saving the protocol.

   NOTE: Bio-Rad Laboratories does not guarantee that running a protocol written in the Protocol AutoWriter window will always result in a PCR product.

5 Plates

Read this chapter for information about creating and editing plate files:

- Plate Editor window (page 33)
- Select Fluorophores window (page 35)
- Well loading controls (page 36)
- Experiment settings window (page 38)
- Well Groups Manager window (page 40)
- Plate Spreadsheet View window (page 42)

Plate Editor Window

A plate file contains run parameters, such as scan mode and fluorophores, and well contents and instructs the instrument about how to analyze the data. Open the Plate Editor window to create a new plate or to edit the plate currently selected in the Plate tab. Once a plate file is created or edited in the Plate Editor, click **OK** to load the plate file into the Experiment Setup window and run it.

To run an experiment, you must load at least one well with sample type and fluorophore.

> TIP: Click the **Plate Loading Guide** button to open the Plate Loading Guide window from the toolbar for a quick overview of instructions to load a plate.

Opening the Plate Editor

To open the Plate Editor window (Figure 25), follow one of these options:

- To create a new plate, select **File > New > Plate**, or click the **Create New** button in the Plate tab (page 19)
- To open an existing plate, select **File > Open > Plate**, or click the **Open Existing** button in the Plate tab (page 19)
- To edit the current plate in the Plate tab, click the **Edit Selected** button in the Plate tab (page 19)
- To open the plate associated with a data file in the Data Analysis window (page 53), click **View/Edit Plate** on the toolbar

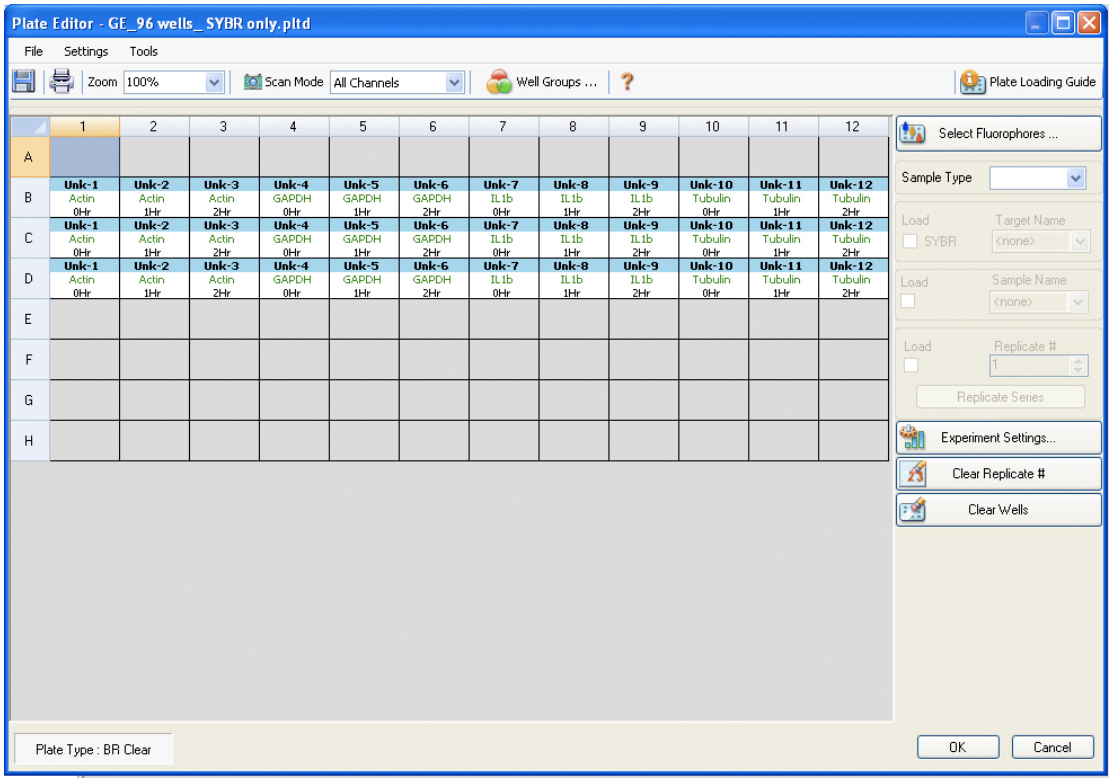


Figure 25. Plate Editor window.

Plate Size and Type

The software applies these plate settings to all the wells during the experiment:

- **Plate Size.** Select a **Plate Size** under **Settings** menu that represents the size of the reaction module block of your instrument. Choosing the instrument type, from the pull down menu option on the Startup Wizard will change the default plate size loaded in the Plate tab of the Experiment Settings window. In the Plate Editor, select the plate size from the Settings menu (see Table 13 on page 35). Plate size cannot be changed during or after the experiment
- **Plate Type.** Select **Clear Wells** or **White Wells** from the **Plate Type** under the **Settings** menu. Make sure the fluorophore being used in the experiment is calibrated for the selected plate type

  NOTE: CFX instruments are factory calibrated for many fluorescent dye and plate combinations. Calibration is specific to the instrument, dye, and plate type. To calibrate a new combination of dye and plate type on an instrument, select **Tools > Calibration Wizard** (see “Calibration Wizard” on page 99)

Scan Mode

The CFX system excites and detects fluorophores in six channels and uses multiple data acquisition scan modes to collect fluorescence data from during a run.

Select one of these scan modes in the Plate Editor window toolbar:

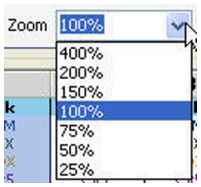
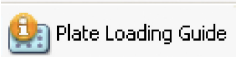
- **All Channels.** Includes channels 1 through 5 on the CFX system
- **SYBR/FAM only.** Includes only channel 1, and provides a fast scan
- **FRET.** Includes only the FRET channel and provides a fast scan

Plate Editor Toolbar

The toolbar in the Plate Editor provides quick access to important plate loading functions:

Table 13 lists the functions available in the Plate Editor toolbar.

Table 13. Toolbar items in the Plate Editor.

Toolbar Item	Name	Function
	Save	Save the current plate file
	Print	Print the selected window
	Zoom	Increase or decrease magnification in plate view
	Scan Mode	Select a scan mode to instruct the instrument what channels to collect fluorescence data from during a run. Select **All Channels** (default), **SYBR/FAM only**, or **FRET**
	Well Groups	Open the Well Groups Manager window and set up well groups for the current plate
	Help	Open the software Help for information about plates
	Plate Loading Guide	Show a quick guide about how to set up a plate and load the wells

Select Fluorophores Window

The Select Fluorophores window lists fluorophores that can be selected to load into the Plate Editor well loading controls. To open the Select Fluorophores window, click the **Select Fluorophores** button on the right side of the Plate Editor.

NOTE: The fluorophores listed depend on the scan mode; when SYBR/FAM only is chosen, only channel 1 fluorophores are shown in the Select Fluorophores window.

NOTE: You cannot add or remove fluorophores in this list; you must calibrate the new fluorophores on an instrument in the Calibration Wizard (page 99). After calibration, the new fluorophore is added to the Select Fluorophore window.

Click the **Selected** check box next to the fluorophore name to add or remove the fluorophores to the list on the right side of the Plate Editor window.

In this example, SYBR is selected from the list of available fluorophores (Figure 26).

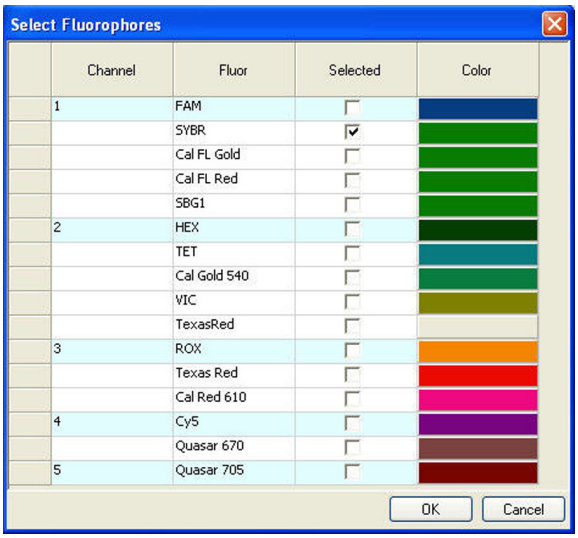


Figure 26. Select Fluorophores window.

- Click the **Color** box next to the fluorophore name and select a new color to represent each fluorophore in the Plate Editor window and Data Analysis charts

  NOTE: Before beginning the run, the software verifies that the fluorophores you specified in the plate are calibrated on that instrument. You cannot run a plate if it includes fluorophores that have not been calibrated on that instrument.

Well Loading Controls

A plate file contains information about the contents of each well loaded with sample for an experiment. After the run, the software links the well contents to the fluorescence data collected during the protocol and applies the appropriate analysis in the Data Analysis window. For example, wells loaded with standard sample type are used to generate a standard curve.

Consider the following guidelines for well contents:

- **Target Name.** One or more targets of interest (genes or sequences) in each loaded well. Each target is assigned to one fluorophore
- **Sample Name.** One identifier or condition that corresponds to the sample in each loaded well, such as 0 hr, 1 hr, or 2 hr

Select a well to load contents into by left clicking with the mouse pointer in the plate view. Hold down the mouse button and drag to select multiple wells. The buttons and lists on the right side of the plate view include all the options needed to load the wells (Table 14).

Table 14. Options for loading the plate and wells in the Plate Editor.

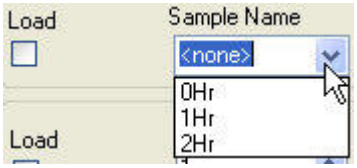
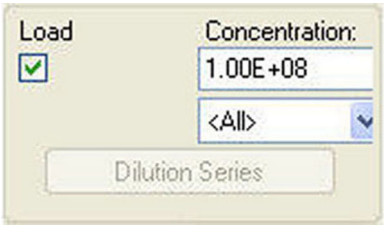
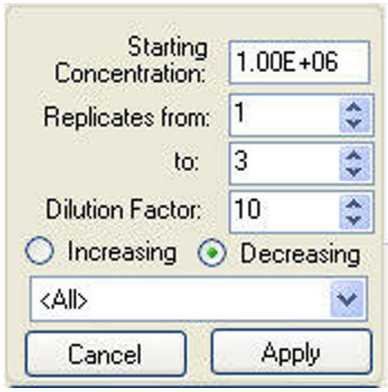
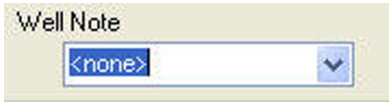
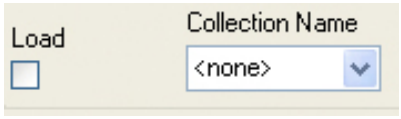
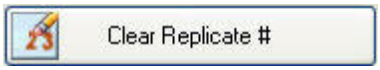
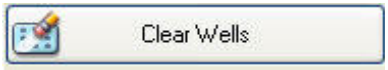
Option	Function
	After selecting wells, the Sample Type must be loaded first. Select a **Sample Type** from the pull-down menu to load it in the selected wells, including Unknown, Standard, NTC (no template control), Positive Control, Negative Control, and NRT (no reverse transcriptase).
	Click a **Load** box to add a fluorophore to the selected wells; each fluorophore corresponds to a target name. To add fluorophores to the Load list, select them in the **Select Fluorophores** window. To distinguish between multiple targets, select a name in the **Target Name** pull- down menu and press the **Enter** key to load the target name in the well. To add a new target name to the pull-down menu in the current plate only, type a name in the pull-down box and press the **Enter** key. To delete a target name, select it, press the **Delete** key, and press the **Enter** key.
	For gene expression analysis or to distinguish between multiple samples, select a **Sample Name** from the pull-down menu to load that sample name in the selected wells. To add a new sample name to the pull-down menu in the current plate, type a new name in the pull-down box and press the **Enter** key. To delete a sample name, select it in the menu, press the **Delete** key on your keyboard, and then press **Enter**.
	To load replicate numbers, selected wells must contain identical well contents. If they do not, the software disables this loading control. Click the **Load** box to add a Replicate # to the selected wells.
	In the **Replicate Series** pane you can apply a replicate series to a set of selected wells. Enter the **Replicate Group Size** by selecting a number that represents the number of samples (wells) in each group of replicates. Select a **Starting Replicate #** to add replicates. You can load replicate groups with replicate numbers progressing from left to right (**Horizontal**), or progressing from top to bottom (**Vertical**).

Table 14. Options for loading the plate and wells in the Plate Editor.

Option	Function
	Enter a concentration to the selected wells with standard sample type by editing or typing a number in the **Concentration** box. To apply the concentration to one fluorophore in the well, select a single fluorophore from the pull-down menu (**<All>**) under the concentration box. To delete a concentration, select it, press the **Back Space** key on your keyboard, and then press **Enter.** Select multiple wells with a Standard sample type to activate the **Dilution Series** button.
	Click the **Dilution Series** button to enter a dilution series for the concentration of Standard samples and load a standard curve. Enter the **Starting Concentration** for the dilution series, the **Replicates from** (starting replicate number) and **to** (ending replicate number), and the **Dilution Factor** (amount to change the concentration with each replicate group). Select **Increasing** for a dilution series that increases, or select **Decreasing** for a dilution series that decreases. Finally, select the fluorophore used for the dilution series from the pull-down menu, and click **Apply.**
	Select **Tools > Show Well Notes** to show this pane. Enter notes about one or more wells by selecting the wells and typing the notes in the pull-down menu. Any notes you add appear in the spreadsheet on the Quantitation Data tab (page 68).
	Select **Tools > Show Collection Name** to show this pane. Enter sample collection information about one or more wells by selecting the wells and typing a collection name in the pull-down menu. Any collection name you add to wells appear in the Gene Expression Analysis window and enables sample grouping options.
	Click the **Experiment Settings** button to open the Experiment Settings window to manage the lists of Targets and Samples, and to set up a gene expression experiment.
	Click the **Clear Replicate #** button to clear the replicates numbers in the selected wells.
	Click the **Clear Wells** button to permanently clear all content in the selected wells

Experiment Settings Window

To open the Experiment Settings window, follow one of these options:

- In the Plate Editor, click the **Experiment Settings** button
- While analyzing data in the Data Analysis window, click the **Experiment Settings** button in the Gene Expression tab

Open the Experiment Settings window to view or change the list of Targets and Samples (Figure 27) or to set the gene expression analysis sample group to be analyzed if **Collection Names** have been added to the wells.

- **Targets.** A list of target names for each PCR reaction, such as a genes or sequences of interest. Click the Reference column to assign reference genes in an experiment
- **Samples.** A list of sample names that indicate the source of the target, such as a sample taken at 1 hour (1 hr), or taken from a specific individual (mouse1). Click the **Control** column to assign the control condition for an experiment

Figure 27 shows the Targets tab with the analysis settings shown.

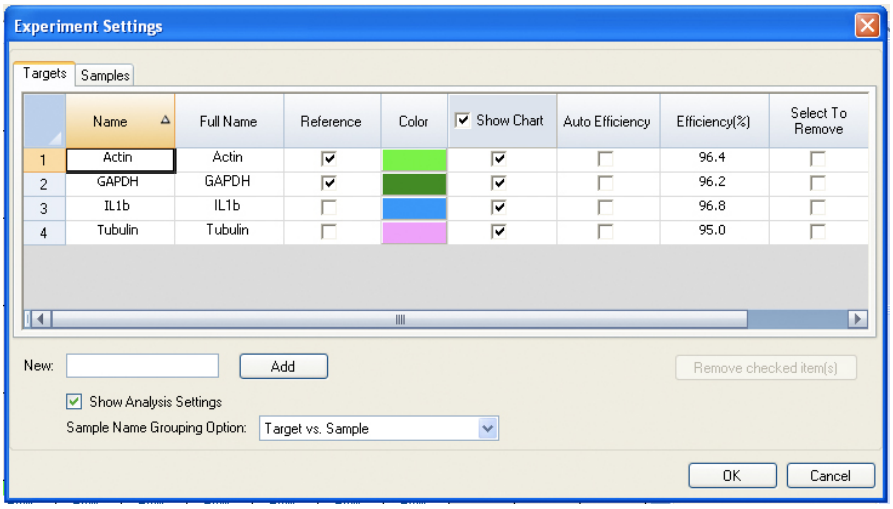


Figure 27. Targets tab in Experiment Settings window.

Figure 28 shows the Sample Tab with the analysis settings shown.

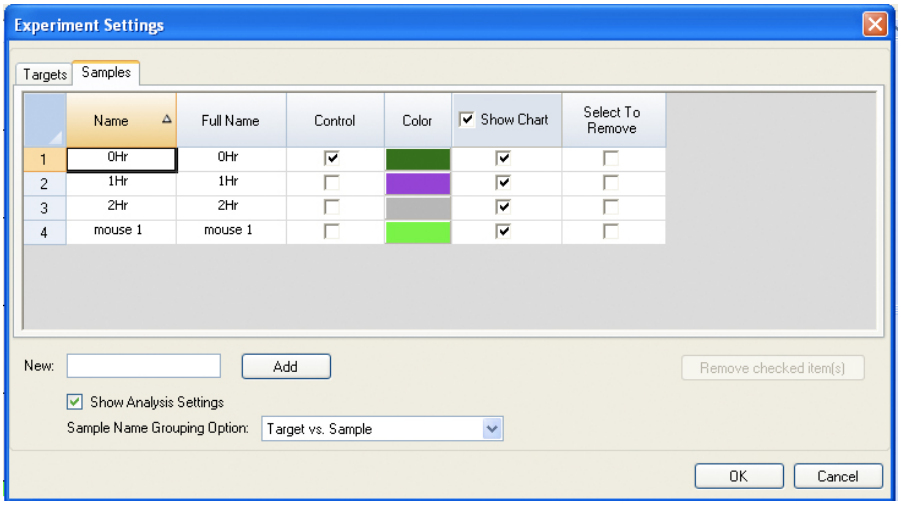


Figure 28. Samples tab in Experiment Settings window.

To adjust the lists in these tabs, use the following functions:

- Add a target or sample name by typing a name in the **New** box and clicking **Add**
- Remove a target or sample name from the list by clicking the **Select to Remove** box for that row, and then clicking the **Remove checked item(s)** button
- Select the target as a reference for gene expression data analysis by clicking the box in the **Reference** column next to the name for that target
- Select the sample as a control sample for gene expression data analysis by clicking the box in the **Control** column next to the name for that sample

Click the **Show Analysis Settings** box in the Experiment Settings window to view or change analysis parameters applied in the Gene Expression tab.

To adjust target parameters:

- Click a cell in the **Color** column to change the color of the targets graphed in the Gene Expression chart
- Enter a number for the efficiency of a target. The software will calculate the relative efficiency for a target using **Auto Efficiency** if the data for a target includes a standard curve. Alternatively, type a previously determined efficiency

To adjust the settings for a sample in the Samples tab:

- Click a color in the **Color** column to change the color of the samples graphed in the Gene Expression chart
- Click a box in the **Show Graph** column to show the sample in the Gene Expression chart using a color that is selected in the **Color** column

Sample Name Grouping Option

Loading **Collection Names** in the wells enables samples to be analyzed in one of four configurations defined by the Sample Name Grouping Option. These options are available from the pull down menu in the Experiment Settings tab.

- Target vs. Sample
- Target vs. Collection
- Target vs. Sample_Collection
- Target vs. Collection_Sample

Well Groups Manager Window

Well groups divide a single plate into subsets of wells that can be analyzed independently in the Data Analysis window. Once well groups are set up, select one in the Data Analysis window to analyze the data in an independent group. For example, set up well groups to analyze multiple experiments run in one plate or to analyze each well group with a different standard curve.

NOTE: The default well group is **All Wells**.

Create Well Groups

To create well groups in the Well Groups Manager window, follow these instructions:

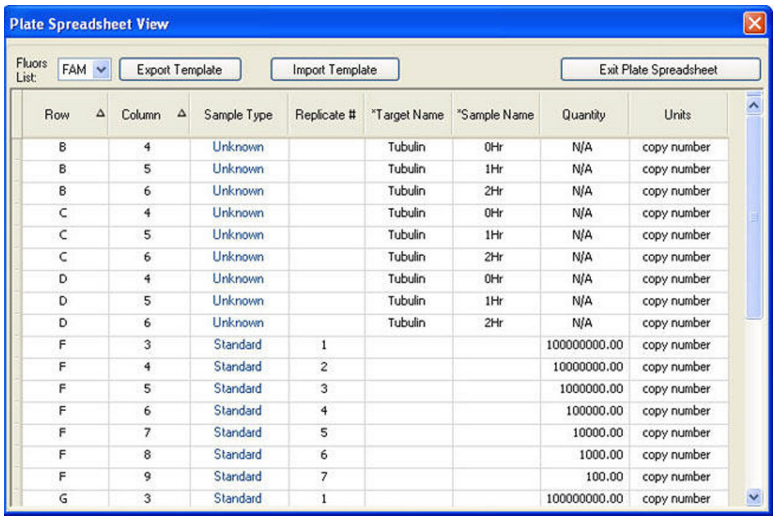
1. Click the **Well Groups** button in the toolbar of the Plate Editor.
2. Click **Add** to create a new group. The pull-down menu shows the group name as **Group 1** for the first group.

3. Select the wells that will compose the well group in the plate view by clicking and dragging across the group of wells. Selected wells turn blue in color (Figure 29).
4. (Optional) Change the name of the group by selecting the group name in the pull-down menu and typing a new name.
5. (Optional) Create more well groups by repeating steps 1 and 2.
6. (Optional) Delete well groups by selecting the group name in the pull-down list, and clicking the **Delete** button.
7. Click **OK** to finish and close the window, or click **Cancel** to close the window without making changes.

Figure 29. Color of wells in the Well Group Manager window.

Plate Spreadsheet View Window

The Plate Spreadsheet View window shows the contents of a plate in the Plate Editor. Open the Plate Spreadsheet View window (Figure 30) by selecting **Tools > Show Spreadsheet View** in the Plate Editor menu bar.



Row	Column	Sample Type	Replicate #	*Target Name	*Sample Name	Quantity	Units
B	4	Unknown		Tubulin	0Hr	N/A	copy number
B	5	Unknown		Tubulin	1Hr	N/A	copy number
B	6	Unknown		Tubulin	2Hr	N/A	copy number
C	4	Unknown		Tubulin	0Hr	N/A	copy number
C	5	Unknown		Tubulin	1Hr	N/A	copy number
C	6	Unknown		Tubulin	2Hr	N/A	copy number
D	4	Unknown		Tubulin	0Hr	N/A	copy number
D	5	Unknown		Tubulin	1Hr	N/A	copy number
D	6	Unknown		Tubulin	2Hr	N/A	copy number
F	3	Standard	1			100000000.00	copy number
F	4	Standard	2			10000000.00	copy number
F	5	Standard	3			1000000.00	copy number
F	6	Standard	4			100000.00	copy number
F	7	Standard	5			10000.00	copy number
F	8	Standard	6			1000.00	copy number
F	9	Standard	7			100.00	copy number
G	3	Standard	1			100000000.00	copy number

Figure 30. Plate Spreadsheet View window.

Open the spreadsheet view to import or export the well contents to Excel or to another tab-delimited format:

- Click **Import Template** to import well contents from a comma delimited file
- Click **Export Template** to export well contents in Excel file (.csv format)

Sort or edit a column by selecting it and using these methods:

- Sort the spreadsheet according to the data in one column by clicking the diamond next to a column name
- Edit the contents of a column that has an asterisk (*) at top by clicking and typing in each well

  NOTE: Select the units for the standard curve data in the Quantity column by opening the Plate Editor and selecting **Settings > Units** in the menu bar. After the plate runs, the data from these standards appear in the Standard Curve chart of the Quantitation tab (Data Analysis window) with the units you select. Open the spreadsheet view to import or export the plate contents to Excel or another tab-delimited format.

Right-click on the spreadsheet to select one of these options from the right-click menu:

- **Copy.** Copy the entire spreadsheet
- **Copy as Image.** Copy the spreadsheet as an image file
- **Print.** Print the spreadsheet
- **Print Selection.** Print only the selected cells
- **Export to Excel.** Export the file as an Excel formatted file
- **Export to Text.** Export the file as a text file
- **Find.** Find text in the spreadsheet
- **Sort.** Sort the spreadsheet by selecting up to three columns of data in the Sort window

6 Stand-Alone Operation

Read this chapter for information about running the CFX system in stand-alone mode:

- C1000™ thermal cycler (page 43)
- Creating a new experiment (page 45)
- Exporting data for analysis (page 49)
- Creating a data file (page 51)

C1000 Thermal Cycler

The CFX system can run real-time PCR experiments without a computer. You can export the fluorescence data acquired during a run using the USB thumb key. You can also choose to have the data emailed directly to you if the C1000 base is attached to the internet and the email functionality has been configured (see the C1000 thermal cycler Instruction Manual for information on how to configure the email settings). The data requires CFX™ Manager software for analysis.

The control panel on the C1000™ thermal cycler provides access to all the functions needed to run the instrument. Figure 31 shows the components of the control panel:

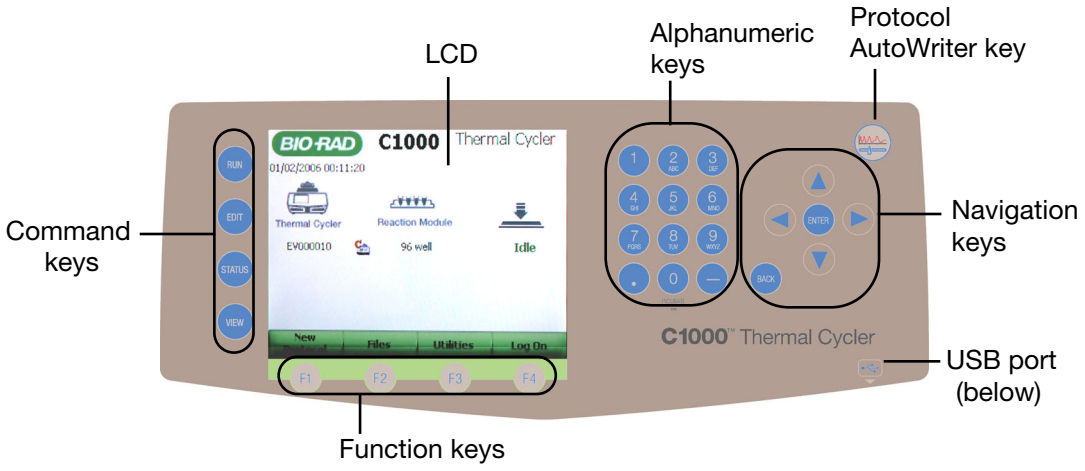


Figure 31. The C1000 thermal cycler control panel.

The control panel contains five sets of keys with the functions listed in table Table 15:

Table 15. Functions of keys on control panel.

Key	Function
COMMAND KEYS	
RUN	Select and run a protocol
EDIT	Select and change protocol
STATUS	View the status of one or more running protocols
VIEW	Switch between graphic and text view of a protocol
FUNCTION KEYS	
F1, F2, F3, or F4	Function key buttons' names and functions change on each screen
ALPHANUMERIC KEYS	
1 through 9	Enter numbers or letters of the alphabet. Press a key multiple times to switch to each associated letter
0, INCUBATE	Insert a zero, ∞ (infinity), or start instant incubation
decimal point (.)	Enter a decimal point
minus sign (–)	Enter a minus sign
Protocol AutoWriter	
Protocol AutoWriter key	Launch the Protocol AutoWriter
NAVIGATION KEYS	
RIGHT arrow	Move cursor to the right
LEFT arrow	Move cursor to the left
UP arrow	Move cursor up
DOWN arrow	Move cursor down
ENTER	Confirm a setting
BACK	Cancel a function. Delete a letter, number, or word

Main Menu

When the CFX system starts, it runs a self-test to verify proper functions and then displays the main menu. The main menu provides access to all system operations, displays the date and time, the name of the logged-in user, the system status, the type of reaction module and thermal cycler name, and any attached S1000™ thermal cyclers.

NOTE: The C1000 thermal cycler stores up to 20 real-time PCR experiment runs using a date/time stamp on the runs. When 20 runs are stored on the C1000, older run data is deleted when a new run is stored.

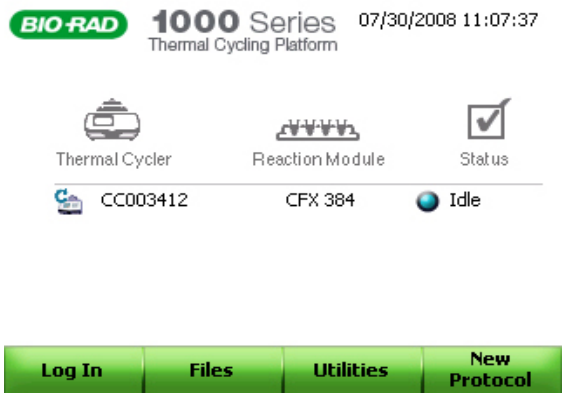


Figure 32. Start-up screen on the front panel.

To initiate the functions in the main menu, press the associated function keys (F1 through F4):

- **Log In (F1)**. Log in to the C1000 thermal cycler. Once you log in the button name changes to Log Off
- **Files (F2)**. View the files and folders in the file library
- **Utilities (F3)**. Open the Utilities menu
- **New Protocol (F4)**. Create a new protocol

Creating a New Experiment

1. Select **New Protocol** (F4) in the start up screen to begin (Figure 33).

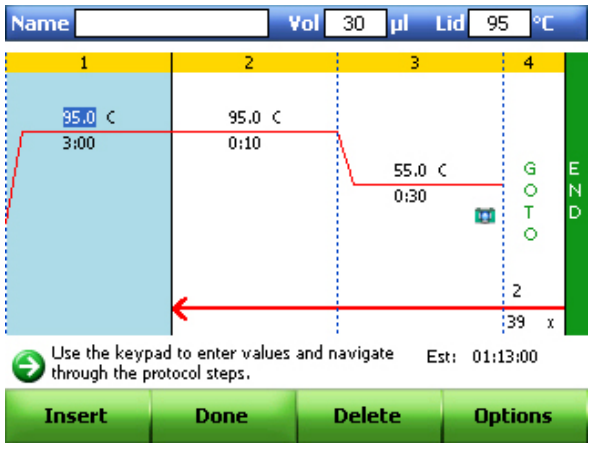


Figure 33. Default real-time PCR protocol.

2. To change the target temperature and the hold time in a temperature step, press the arrow keys to navigate between steps and to select a parameter (temperature or time). Press the alphanumeric keys to enter a new number for each parameter you highlight.

   TIP: Connect a computer mouse via a USB port on the C1000 chassis to navigate.

3. (Optional) To insert a new step, select the **Insert** (F1) button. To delete a step, select the **Delete** (F3) button (Figure 33).

4. (Optional) To change step options, select the **Options** (F4) button (Figure 33). In the **Step Options** window, select a parameter to change, including the temperature and time of the step, or add/remove a plate read to the step (Figure 34).

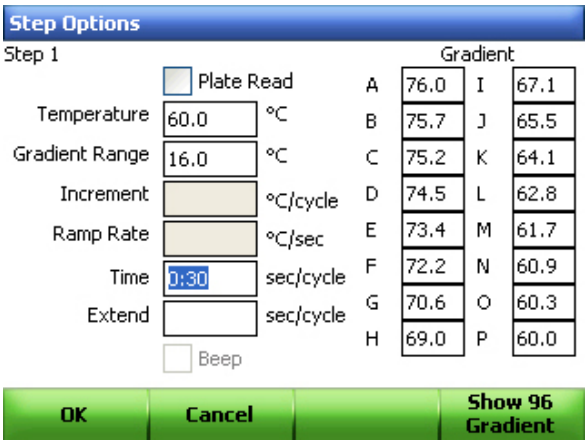


Figure 34. Step Options window.

NOTE: Press the alphanumeric keys to enter a **Gradient Range** spanning from 1 to 24°C.

5. The **GOTO** step instructs the thermal cycler to repeat a set of steps in a loop to create the cycles in the PCR experiment. Select a **GOTO** step; press the arrow keys to select and then edit the step number in a **GOTO** step or to change the number of repeats.

6. (Optional) To change the default sample volume, select the sample volume box (**Vol**) (Figure 33 on page 45). Use the alphanumeric keys to enter a new sample volume in microliters. The sample volume you enter determines the temperature control mode that is used during a run.

7. (Optional) To change the default lid temperature, select the lid temperature box (**Lid**) by pressing the arrow keys (Figure 33 on page 45). Use the alphanumeric keys to enter a new temperature. The default lid temperatures for the CFX96 modules is105°C.

   NOTE: Heating the lid prevents condensation in the sealed reaction vessels.

8. When creating a new protocol, you have the option to save it with a name. Use the arrow keys to navigate to the Protocol **Name** box and then press the alphanumeric keys multiple times to enter a letter or number to type a new protocol name.

9. Press **ENTER** to accept the name.

Running the Protocol

1. To begin the run, click **Done** (F2) in the Protocol window (Figure 33 on page 45).

   TIP: Alternatively, click the **RUN** command key to start the run without saving or editing the name of the protocol.

2. Enter a protocol name if you have already not done so, or edit the name previously created in the Protocol window. Use arrow keys to select a destination folder (Figure 35).

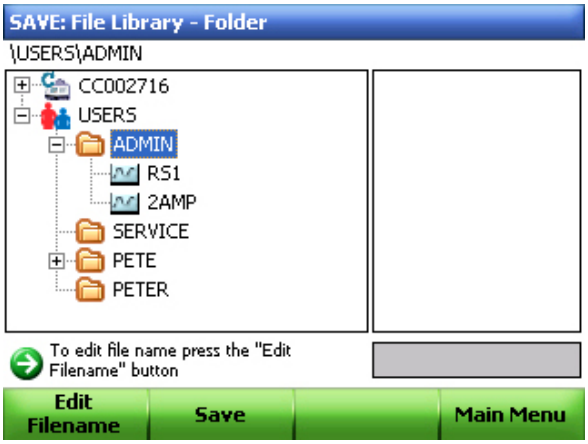


Figure 35. Saving a protocol.

3. Click **Edit Filename** (F1). Type a new name in the box and click **Save** (F2) (Figure 36).

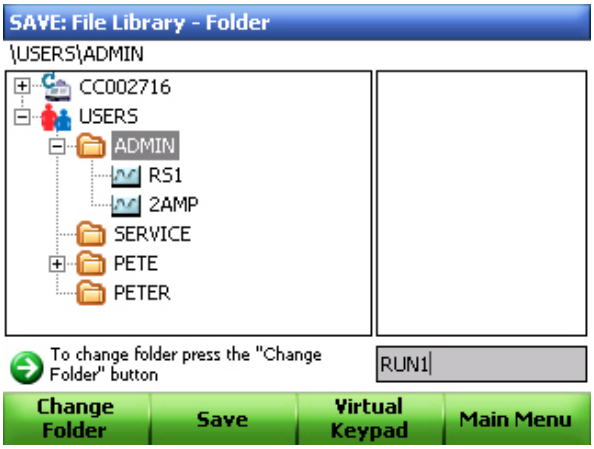


Figure 36. Entering a protocol name.

4. Click **Run** (F2) to continue and run the protocol (Figure 37).

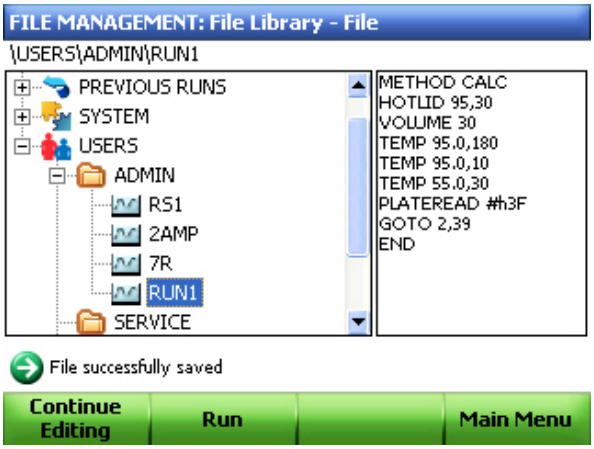


Figure 37. Protocol successfully saved.

5. Edit the **Sample Volume** and **Lid Temperature.** A **Sample ID** or **User** can also be recorded for the run (Figure 38). Click **OK** (F1) to proceed.

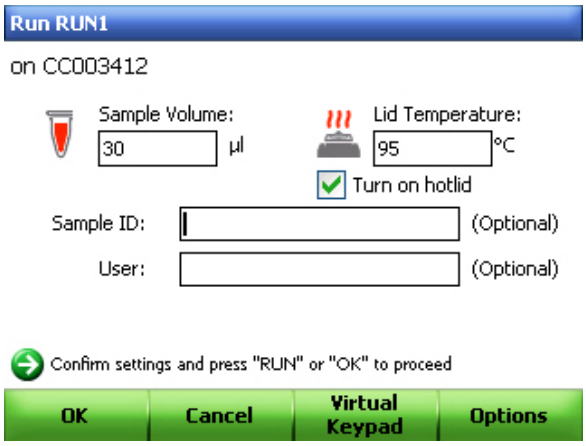


Figure 38. Editing sample volume and lid temperature.

6. Select a **Scan Mode** to collect fluorescence data during a run (Figure 39).

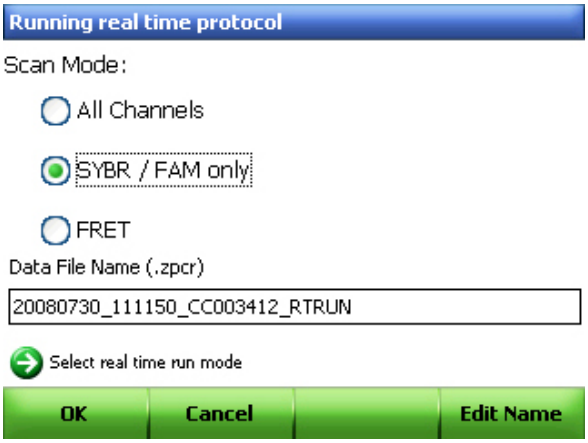


Figure 39. Scan mode and data file name.

Scan modes detect calibrated fluorophores in these channels:

- **All Channels.** Collects data from channels 1 through 5 on the CFX96 and CFX96 Deep Well systems
- **SYBR/FAM.** Collects data only from channel 1 and provides a fast scan
- **FRET.** Collects data only from the FRET channel and provides a fast scan

7. A default stand-alone data file name is created prior to the run. If you wish to change the name, use the arrow keys to navigate to the **Data File Name** box, then press the alphanumeric keys to enter a letter or number to type a new data file (.zpcr) name.

8. Click the **OK** (F1) button to start the run.

Running a Previously Saved Protocol

- To change an existing protocol, press the **EDIT** key to open the file library and select a protocol to edit

- To run an existing protocol, press the **RUN** command key and select a previously saved protocol from the file library

Monitoring a run

When a run begins, the run status window appears. Review the information in this window to monitor the progress of a run.

- **Status.** Press the **STATUS** command key to check the current status of the protocol, pause the run, cancel a run, skip a step, or access the main menu (Figure 40)
- **Time Status.** Press the **VIEW** command key to see a full-screen count-down timer for the protocol. Press the **VIEW** key again to switch back to the Status screen

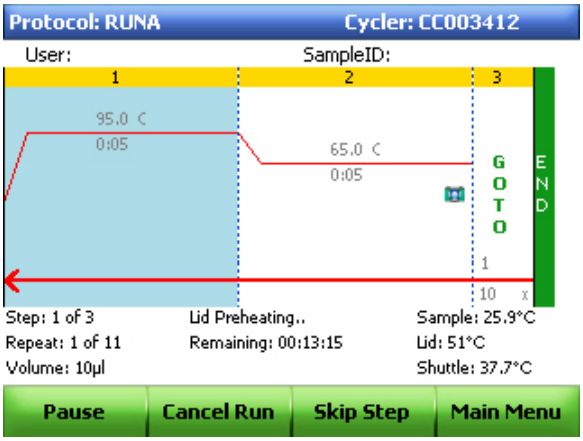


Figure 40. Monitoring run status.

Exporting Data for Analysis

When the run is finished, the fluorescence data need to be transferred to a computer running CFX Manager software for analysis. The stand-alone data file is automatically saved to the **RT_DATA** folder located in the **SYSTEM** folder (Figure 41).

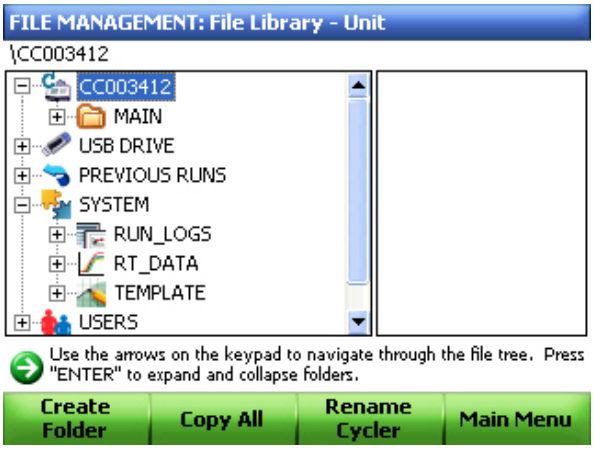


Figure 41. RT_DATA folder stores real-time PCR runs.

If a USB key has been placed in a USB key port on the C1000 thermal cycler, the data (.zpcr) will automatically be saved to the root directory of the USB key.

If a USB key is not in the thermal cycler at the end of the run, follow these instructions:

1. Press the **Files** (F2) button on the main screen to access the file folders.
2. Navigate to the RT_DATA folder and then press the right arrow key to open the folder.
3. Select the file using the up and down arrow keys.
4. Press **Export File** (F1) to export the run data (.zpcr) to the USB key (Figure 42).

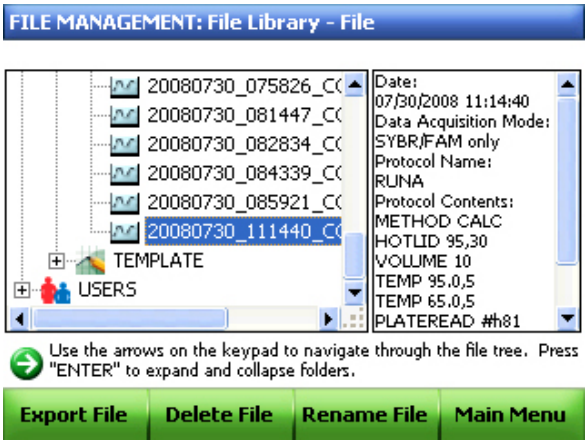


Figure 42. Exporting stand-alone run data to a USB key.

5. Click **Yes** (F1) to confirm the export.

You can choose to email your data to you directly from the C1000 thermal cycler after the run completes by configuring the email settings (see the C1000 thermal cycler instruction manual for information on configuring the email settings).

To send an email with attached data (.zpcr) at the end of a run, follow these instructions:

1. Select **Options** (F4) in the Run information screen (Figure 38 on page 48).
2. Using the arrow keys select the **Send email notification** option.
3. Click **OK** (F1) to return to the Run information screen.
4. Use the arrow keys to navigate to the **Email Address** box and then use the alphanumeric keys to enter the email address.

5. Click **OK** (F1) to continue to run the assay.

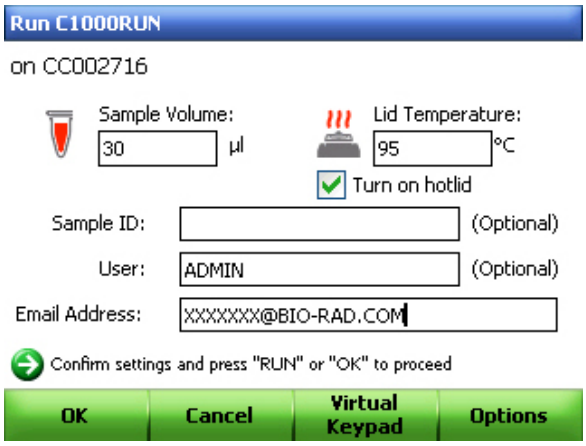


Figure 43. Confirming export to USB key.

Creating a Data File

The stand-alone run data (.zpcr) data need to be converted into a data file (.pcrd) by CFX Manager software to be analyzed. To create a data file from a stand-alone run:

1. Click and drag the .zpcr file from the USB key directory over the main software window, or **Select File > Open > Stand-alone Run** from the main software window menu options to select the file name.

2. In the **Run File Processor** window click the **Select Plate** button to import the name of the plate file the software will use to create the data file (Figure 44).

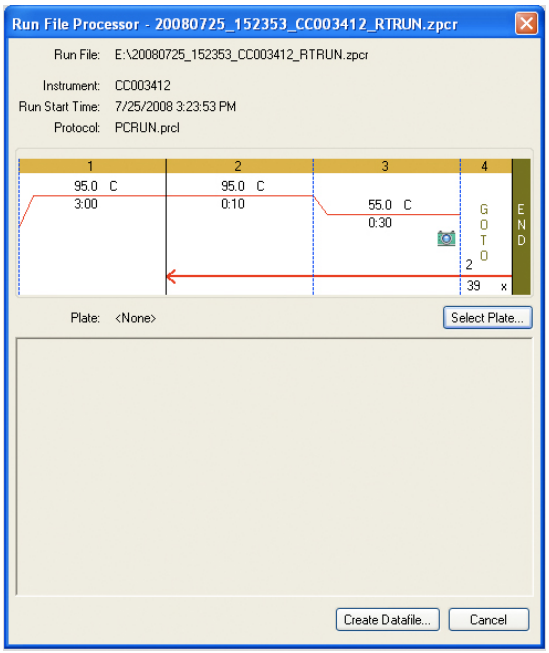


Figure 44. Assigning a plate file.

NOTE: CFX Manager software checks the scan mode and plate size for the plate file; these must match the current run settings that were started during the experiment. Load a Quick Plate file to quickly access data from all the wells.

7 Data Analysis Overview

Read this chapter for information about data analysis:

- Data analysis precautions (page 53)
- Data Analysis window (page 54)
- Quantitation tab (page 57)
- Data analysis settings (page 58)
- Well selectors (page 60)
- Charts (page 62)
- Spreadsheets (page 62)

Data Analysis Precautions

There are many ways in which the data generated from the system can be interpreted. Occasionally, the data can be interpreted incorrectly. In order to prevent data misinterpretation, please follow these guidelines when inferring a result from the data:

1. Confirm that the sample data is correct. If a barcode was entered, check that the barcode of the assay or plate matches that of the sample.
2. Confirm that the appropriate assay was run using the appropriate protocol.
3. Check that the controls were included in the assay and generated the expected result.
4. Confirm that the assay was run to completion.
5. Check that the appropriately diluted samples were loaded into the correct wells and assigned the correct dye, Be sure that the samples were run in duplicate at a minimum.
6. Check to optimize an examination procedure in order to improve its performance characteristics.
7. Care should be taken to note if any variable in the protocol was modified as this could alter the call for the assay. These variables can include changes due to:
   - Manually adjusting the baseline
   - Manually adjusting the threshold
   - Cq determination mode
   - Removal of outliers
   - Removal or changes to QC check rules

Data Analysis Window

During data analysis, changing the way the data are displayed by changing the contents of wells in the Plate Editor never changes the fluorescence data that were collected from each well during the run. Once the module collects fluorescence data you cannot delete those data, but you can choose to remove data from view and analysis.

To change the content of wells after a run, open the Plate Editor by clicking the **Edit/View Plate** button at the top of the Data Analysis window.

> TIP: You can add or edit information about the contents of the well before, during, or after you run the real-time PCR experiment. You must assign the scan mode and plate size before the run, and these parameters cannot change after the run.

CFX Manager™ software processes real-time PCR data automatically at the end of each run, and opens the Data Analysis window to display these data. Choose one of these methods to open existing data files in the Data Analysis window:

- Drag a data file (.pcrd extension) over the main software window and release it
- Select **File > Open > Data File** in the main software window to select a file in the Windows browser
- Click the **Data Analysis** button in the main software window toolbar to select a file in the Windows browser
- Select **File > Recent Data Files** to select from a list of the ten most recently opened data files

The Data Analysis window displays up to nine tabs (Figure 45). Each tab shows the analyzed data for a specific analysis method:

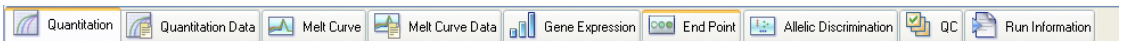


Figure 45. All the tabs that can display in the Data Analysis window.

The software only displays a tab in the Data Analysis window if the data are collected in the run and are available for that type of analysis.

Data Analysis Toolbar

The toolbar in the Data Analysis window provides quick access to important data analysis functions.Table 16 lists the functions of buttons in the toolbar.

Table 16. Toolbar in the Data Analysis window.

Toolbar button	Name	Function
	Save	Save the current data file
	Print	Print the selected window
	Trace Style	Open Trace Style window

Table 16. Toolbar in the Data Analysis window. (continued)

Toolbar button	Name	Function
	Report	Open a Report for the current data file
	View/Edit Plate	Open the Plate Editor to view and edit the contents of the wells
	Well Groups...	Select a well group name from the pull-down menu. The default selection is All Wells
	Help	Open the software Help site for more information about data analysis

Data Analysis Menu Bar

Table 17 lists the functions of items in the menu bar.

Table 17. Menu bar items in Data Analysis window.

Menu Item	Command	Function
File	Save	Save the file
	Save As	Save the file with a new name
	Repeat Experiment	Extract the protocol and plate file from the current experiment to rerun it
	Exit	Exit the Data Analysis window
View	Run Log	Open a Run Log window to view the run log of a data file
Settings	Analysis Mode	Select Baseline Subtraction method for the selected well groups in the data
	C(t) Determination Mode	Select Regression or Single-Threshold mode to determine how C(t) values are calculated for each trace
	Baseline Thresholds	Open the Baseline Thresholds window to adjust the baseline or the threshold
	Trace Styles	Open the Trace Styles window
	View/Edit Plate	Open the Plate Editor to view and edit the plate
	Mouse Highlighting	Turn on or off the simultaneous highlighting of data with the mouse pointer TIP: If the Mouse Highlighting is turned off, then hold down the Control key to temporarily turn on the highlighting
	Display Threshold Values	Display the value of the threshold line in the chart
Tools	Reports	Open the Report for this data file

Table 17. Menu bar items in Data Analysis window. (continued)

Menu Item	Command	Function
	Import Fluorophore Calibration	Select a calibration file to apply to the current data file
	Replace Plate	Replace the current plate file in the data analysis
	Export All Data Sheets to Excel	Export all the spreadsheet views from every tab to a separate Excel formatted file
Help		Open software Help for more information about data analysis

Quantitation Tab

Each tab in the Data Analysis window displays data in charts and spreadsheets for a specific analysis method, with a well selector to select the data you want to show. The Data Analysis window opens with the Quantitation tab (Figure 46) in front. The **Amplification** chart data in this tab should be used to determine the appropriate analysis settings for the experiment.

NOTE: The **Amplification** chart shows the relative fluorescence (RFU) for each well at every cycle. Each trace in the chart represents data from a single fluorophore in one well.

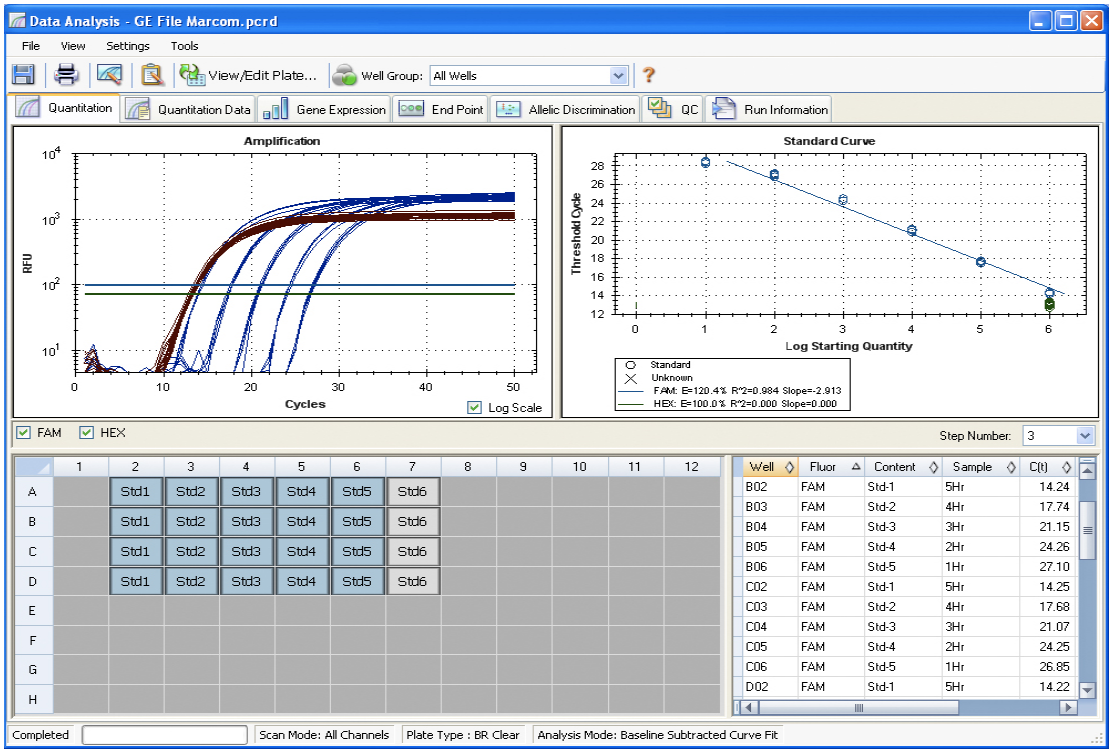


Figure 46. Layout for the Quantitation tab in the Data Analysis window.

NOTE: The software links the data in the panes of each data analysis tab. For example, highlighting a well by placing the mouse pointer over the well in the well selector view highlights the data in all the other panes.

Step Number Selector

The CFX system can acquire fluorescence data at multiple protocol steps; the software maintains the data acquired at each step independently. The software displays the **Step Number** selector below the Standard Curve chart on the Quantitation tab whenever a protocol contains more than one data collection step. When you select a step, the software applies that selection to all the data that are shown in the Data Analysis window. Figure 47 shows the data collection step number is **3** for all the data.

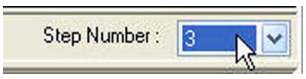


Figure 47. Step Number selection in the Data Analysis window.

Viewing Well Groups in Data Analysis

Wells in the plate can be grouped into subsets for independent analysis using well groups. When you create well groups in the **Well Groups Manager** window in the Plate Editor (page 40), group names appear in the Data Analysis window within the Well Groups list on the toolbar.

By default, the well group **All Wells** is selected when the Data Analysis Window is first opened, with the data in all wells with content shown in the charts and spreadsheets.

Figure 48 shows Group 2 selected in the Well Groups menu. Only the wells in that well group appear loaded in the well selector and data for these wells only are included in the data analysis calculations.

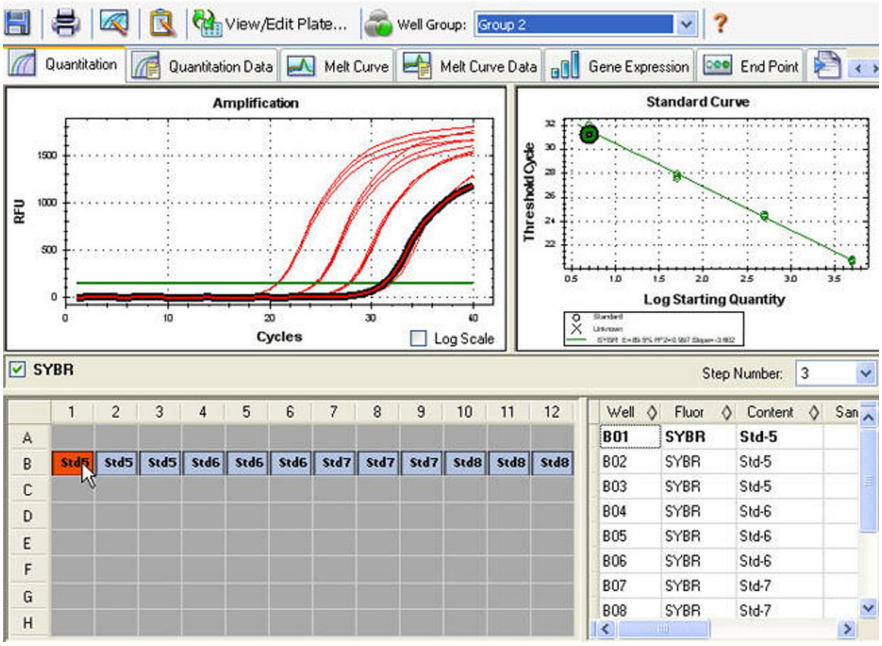


Figure 48. Data Analysis window with Group 2 selected.

Data Analysis Settings

The **Amplification** chart data in the Quantitation tab shows the relative fluorescence (RFU) for each well at every cycle. Each trace in the chart represents data from a single fluorophore in one well. These data are used to determine C(t) values for each well on a per fluorophore basis. The software uses one of two modes to determine C(t) values:

- **Regression.** This mode applies a multivariable, nonlinear regression model to individual well traces and then uses this model to compute an optimal C(t) value
- **Single Threshold.** This mode uses a single threshold value to calculate the C(t) value based on the threshold crossing point of individual fluorescence traces

Adjusting the Threshold

In Single-Threshold mode, adjust the threshold for a fluorophore by clicking on the threshold line in the Amplification chart and moving the mouse pointer vertically. Alternatively, specify an exact crossing threshold for the selected fluorophore by following these instructions:

1. Select **Settings > Baseline Thresholds** in the menu bar to open the Baseline Thresholds window.
2. Adjust the crossing threshold (Figure 49) for the fluorophore by clicking **User Defined** and entering a threshold number.

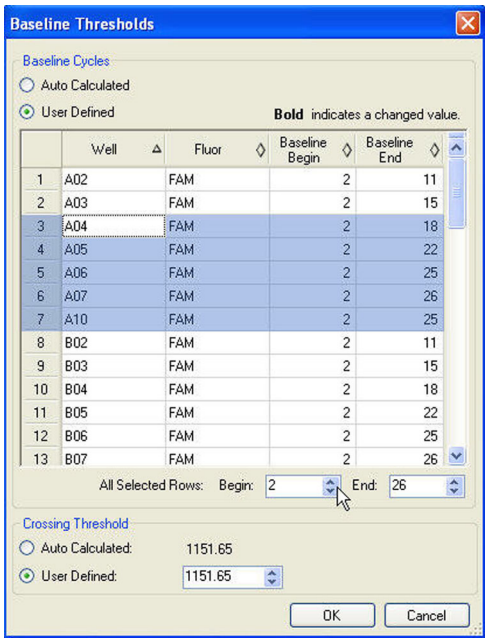


Figure 49. Baseline Thresholds window.

3. Click **OK** to confirm the change and close the window.

Baseline Settings

The software automatically sets the baseline individually for each well. Once you select the wells for analysis, check the baseline settings in these wells. Open the Baseline Thresholds window (Figure 49) to change the default baseline for selected wells. To open this window:

1. Select **Settings > Baseline Thresholds** to open the Baseline Thresholds window.

To adjust the begin and end baseline cycle for each well:

1. In the Baseline Cycles pane, select one or more wells by clicking the row number, clicking the top left corner to select all wells, holding down the Control key to select multiple individual wells, or holding down the shift key to select multiple wells in a row.
2. Adjust the **Baseline Begin** cycle and **Baseline End** cycle for all selected wells or change the **Begin** and **End** cycle number at the bottom of the spreadsheet (Figure 49).
3. Click **OK** to confirm the change and close the window.

Select the Analysis Mode

Select the Analysis Mode to determine the method of baseline subtraction for all fluorescence traces. Select **Settings > Analysis Mode** to choose one of these three options:

- **No Baseline Subtraction.** The software displays the data as relative fluorescence traces. Some analysis is not possible in this analysis mode
- **Baseline Subtracted.** The software displays the data as baseline subtracted traces for each fluorophore in a well. The software must baseline subtract the data to determine threshold cycles, construct standard curves, and determine the concentration of unknown samples. To generate a baseline subtracted trace, the software fits the best straight line through the recorded fluorescence of each well during the baseline cycles, and then subtracts the best fit data from the background subtracted data at each cycle
- **Baseline Subtracted Curve Fit.** The software displays the data as baseline subtracted traces, and the software smoothes the baseline subtracted curve using a centered mean filter. This process is performed so that each C(t) is left invariant

Well Selectors

Click the wells in the well selector to show or to hide the data in the charts or spreadsheets throughout the Data Analysis window:

- To hide one well, highlight and click the individual well. To show that well, highlight and click the well again
- To hide multiple wells, click and drag across the wells you want to select. To show those wells, click and drag across the wells again
- Click the top left corner of the plate to hide all the wells. Click the top left corner again to show all wells
- Click the start of a column or row to hide those wells. Click the column or row again to show the wells

Only wells loaded with content (entered in the Plate Editor) can be selected in the well selector, and their color shows if they are selected. As shown in Figure 50, the well selector shows these three types of wells:

- **Selected, loaded wells (blue).** These wells contain a loaded **Unk** (unknown) sample type. The data from these wells appear in the Data Analysis window
- **Unselected, loaded wells (light gray).** These wells contain loaded **Std** and **Pos** sample types. The data from unselected wells do not appear in the Data Analysis window
- **Empty wells (dark gray).** These wells were not loaded in the Plate Editor window

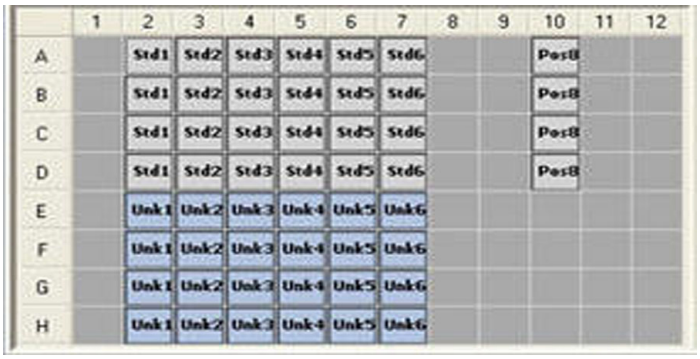


Figure 50. Three well colors appear in a well selector.

Temporarily Exclude Wells from Analysis

RIGHT-CLICK OPTION

1. Right-click on the well in the well selector, on a fluorescence trace, or on a point plotted on the standard curve.
2. Choose **Exclude Well XX from Analysis** from the menu options.

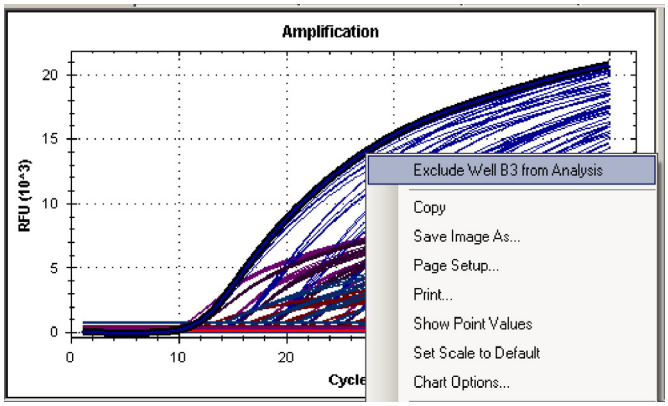


Figure 51. Right-click to exclude a well from analysis.

PLATE EDITOR OPTIONS

1. Click the **View/Edit Plate** button on the toolbar in the Data Analysis window.
2. Select one or more wells in the well selector view.
3. Click **Exclude Wells in Analysis** (Figure 52) to exclude the selected wells. This checkbox is at the bottom of the Plate Editor controls on the right side of the window.

Figure 52. Exclude Wells in Analysis checkbox at bottom of the pane.

4. Excluded wells are marked with an asterisk (*) in the Plate Editor window.

Alternatively, to permanently remove wells from analysis, clear the contents from wells in the Plate Editor by clicking the **Clear Wells** button.

WARNING! You will have to reenter any well content that is cleared.

Charts

Each chart in the Data Analysis window displays the data in a different graph and includes options for adjusting the data. To magnify an area of the chart, select an area by clicking and dragging the mouse. The software resizes the chart and centers it on the selected area.

Common Right-Click Menu Items for Charts

Right-click menu items are available on all charts. Some of the available items are present for all charts, and these items can be used to change how the data are displayed or to easily export the data from a chart (Table 18.)

Table 18. Right-click menu items for charts.

Item	Function
Copy	Copy the chart into the clipboard
Save Image As...	Save the chart image in the selected image file type. Select from these formats: **PNG** (default), **GIF**, **JPG**, **TIF**, or **BMP**
Page Setup...	Preview and select page setup for printing
Print...	Print the chart
Show Point Values	Show the point values when the mouse moves over a point on the chart.
Set Scale to Default	Return to default chart view after magnifying the chart
Chart Options...	Open the Chart Options window to change the chart, including changing the title, selecting limits for the x and y axes, showing grid lines, and showing minor ticks in the axes

NOTE: Menu items that apply to specific charts are described in the next chapter “Data Analysis Windows” (page 65).

Spreadsheets

The spreadsheets shown in Data Analysis include options for sorting and transferring the data. Sort the columns by one of these methods:

- Click and drag a column to a new location in the selected table
- Click the column header to sort the data in Ascending or Descending order

To sort up to three columns of data in the Sort window, follow these steps:

1. Right-click on the spreadsheet to open the menu and select **Sort**.
2. In the Sort window, select the first column title to sort. Sort the data in Ascending or Descending order.
3. Select more than one column title by selecting the title in the pull-down menu. Select **Ascending** or **Descending** to sort the column in that order.
4. Click **OK** to sort the data, or click **Cancel** to stop sorting.

Highlight the data on the associated charts and well selector by holding the mouse pointer over a cell. If you click in the cell, you can copy the contents to paste into another software program.

Common Right-Click Menu Items for Spreadsheets

Right-click any spreadsheet view to select the items shown in Table 19.

Table 19. Right-click menu items for spreadsheets

Item	Function
Copy	Copy the contents of the selected wells to a clipboard, then paste the contents into a spreadsheet such as Excel
Copy as Image	Copy the spreadsheet view as an image file, and paste it into a file that accepts an image file such as text, image, or spreadsheet files
Print...	Print the current view
Print Selection...	Print the current selection
Export to Excel...	Export the data to an Excel spreadsheet
Export to Text...	Export the data to a text editor
Export to XML	Export the data to an XML file
Export to HTML	Export the data to an HRML file
Find...	Search for text
Sort...	Sort the data in up to three columns

8 Data Analysis Windows

Read this chapter for more information about the tabs in the Data Analysis window:

- Quantitation tab (page 65)
- Quantitation Data tab (page 68)
- Melt Curve tab (page 69)
- Melt Curve Data tab (page 70)
- End Point tab (page 70)
- Allelic Discrimination tab (page 72)
- QC tab (page 74)
- Run Information tab (page 74)
- Data file reports (page 75)

Quantitation Tab

Use the data in the Quantitation tab (Figure 46 on page 57) to set the data analysis conditions, including the baseline settings for individual wells and the threshold settings. The Quantitation tab shows data in these four views:

- **Amplification chart.** Shows the relative fluorescence units (RFUs) for each well at every cycle. Each trace in the chart represents data from a single fluorophore in one well
- **Standard curve.** This graph is only shown if the experiment includes wells designated as Sample Type Standard. Shows a standard curve with the threshold cycle plotted against the log of the starting quantity. The legend shows the Reaction Efficiency (E) for each fluorophore in the wells with a standard sample type
- **Well selector.** Selects the wells with the fluorescence data you want to show
- **Spreadsheet.** Shows a spreadsheet of the data collected in the selected wells

Fluorophore Selector

To select the fluorophore data to display in the Quantitation tab charts and spreadsheets, click the fluorophore selector below the Amplification chart (Figure 53). Click the box next to the fluorophore name to show or hide the fluorophore data throughout the data analysis window.

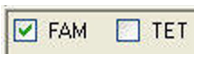


Figure 53. Fluorophore selector with FAM selected.

Trace Styles Window

Open the Trace Styles window (Figure 54) to adjust the appearance of traces in the amplification and melt curve charts in the Quantitation and Melt Curve tabs.

To open this window, follow these steps:

1. Select only one fluorophore in the fluorophore selection boxes.
2. Click the **Trace Styles** button in the Data Analysis toolbar, or select **Settings > Trace Styles** in the Data Analysis menu bar.

Figure 54. Trace Styles window.

Use the tools in the Trace Styles window to adjust appearance of traces and preview the changes in the well selector at the bottom of the window.

- Select a specific set of wells by using the well selector at the bottom of the window. Alternatively, select wells that contain one sample type in the pull-down menu in the **Wells** column
- Click the box in the Color column to select a color for the wells
- Select a symbol from the pull-down menu in the Symbol column
- Click **Show Contents** to show the sample types in each well, or click **Show Symbols** to show the selected Symbols in each well.

Log Scale Option

Click the **Log Scale** box at the bottom of the Amplification chart to view the fluorescence traces in a semi-log scale, as shown in Figure 55.

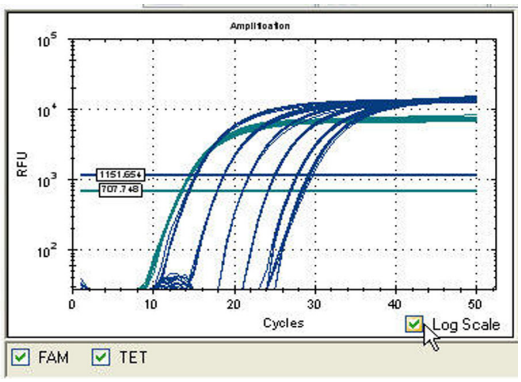


Figure 55. Log Scale option selected in Amplification chart.

Standard Curve Chart

The software creates a Standard Curve chart (Figure 56) in the Quantitation tab if the data include sample types defined as standard (Std) for one fluorophore in the experiment.

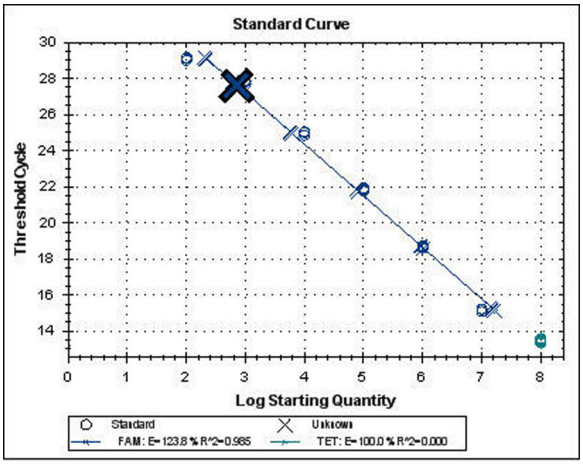


Figure 56. Standard Curve chart.

The Standard Curve chart displays the following information:

- Name for each curve (the fluorophore name)
- Color of each fluorophore
- Reaction efficiency (E). Use this statistic to optimize a multiplex reaction, and equalize the data for a standard curve

  NOTE: The reaction efficiency describes how much of your target is being produced with each cycle in the protocol. An efficiency of 100% means that you are doubling your target with each cycle.
- Coefficient of determination, R^2 (written as R^2). Use this statistic to determine how correctly the line describes the data (goodness of fit)

Chart Right-Click Menu Options

In addition to the common right-click menu options to copy, print, and export charts, Table 20 lists the menu options available only on the Amplification chart.

Table 20. Amplification chart specific right-click menu options.

Menu Option	Function
Show Threshold Values	Display the threshold value for each amplification curve on the chart
Trace Styles...	Open the Trace Styles window to change trace styles that appear on the Quantitation and Melt Curve tabs
Baseline Thresholds...	Open the Baseline Thresholds window to change baseline or thresholds of each fluorophore (changes appear in Amplification chart in Quantitation tab)

Quantitation Data Tab

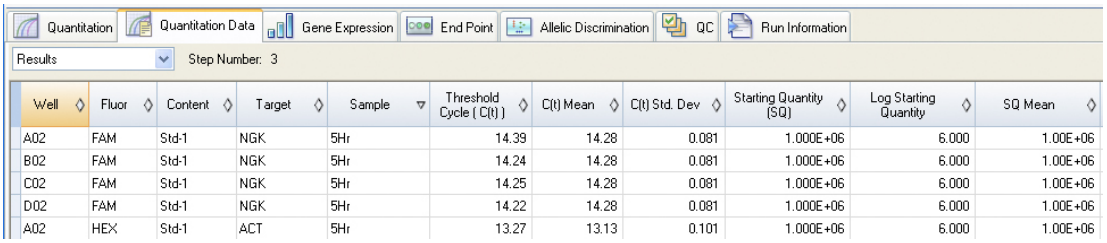
The Quantitation Data tab shows spreadsheets that describe the quantitation data collected in each well. Select one of the three options to show the data in different formats:

- **Results.** Displays a spreadsheet view of the data
- **Plate.** Displays a view of the data in each well as a plate map
- **RFU.** Choose this spreadsheet to show the RFU quantities in each well for each cycle

TIP: Right-click any spreadsheet for options, including the sort option.

Results Spreadsheet

Select a **Results** spreadsheet (Figure 57) to see data for each well in the plate.



Well	Fluor	Content	Target	Sample	Threshold Cycle (C(t))	C(t) Mean	C(t) Std. Dev	Starting Quantity (SQ)	Log Starting Quantity	SQ Mean
A02	FAM	Std-1	NGK	5Hr	14.39	14.28	0.081	1.000E+06	6.000	1.00E+06
B02	FAM	Std-1	NGK	5Hr	14.24	14.28	0.081	1.000E+06	6.000	1.00E+06
C02	FAM	Std-1	NGK	5Hr	14.25	14.28	0.081	1.000E+06	6.000	1.00E+06
D02	FAM	Std-1	NGK	5Hr	14.22	14.28	0.081	1.000E+06	6.000	1.00E+06
A02	HEX	Std-1	ACT	5Hr	13.27	13.13	0.101	1.000E+06	6.000	1.00E+06

Figure 57. Quantitation Data tab with Results spreadsheet selected.

NOTE: All Std. Dev (standard deviation) calculations apply to the replicate groups assigned in the wells in the Plate Editor window. The calculations average the C(t) value for each well in the replicate group.

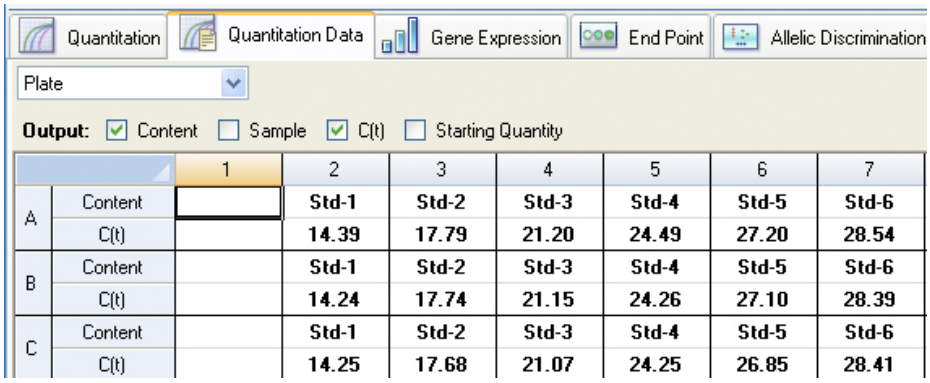
The Results spreadsheet includes the type of information listed in Table 21.

Table 21. Results spreadsheet content.

Information	Description
Well	Well in the plate
Fluor	Fluorophore detected
Content	Sample type and replicate number
Target	Amplification target name (gene)
Sample	Sample description
Threshold Cycle (C(t))	Threshold cycle
C(t) Mean	Mean of the threshold cycle for the replicate group
C(t) Std. Dev	Standard deviation of the threshold cycle for the replicate group
Starting Quantity (SQ)	Estimate of the starting quantity of the target
Log Starting Quantity	Log of the starting quantity
SQ Mean	Mean of the starting quantity
SQ Std. Dev	Standard deviation of the starting quantity
Set Point	Temperature of sample in the well for a gradient step
Sample Note	One round of denaturation, annealing, and extension, or one round of annealing and extension steps in a protocol

Plate Spreadsheet

Select the **Plate** spreadsheet to see a plate map of the data for one fluorophore at a time. Select each fluorophore by clicking a tab at the bottom of the spreadsheet. Figure 58 shows the Plate spreadsheet as plate map.

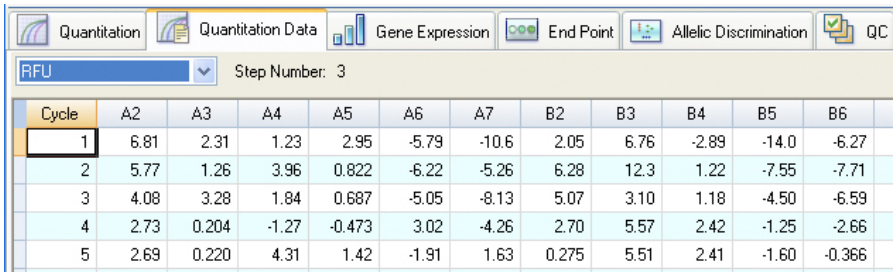


		1	2	3	4	5	6	7
A	Content		Std-1	Std-2	Std-3	Std-4	Std-5	Std-6
	C(t)		14.39	17.79	21.20	24.49	27.20	28.54
B	Content		Std-1	Std-2	Std-3	Std-4	Std-5	Std-6
	C(t)		14.24	17.74	21.15	24.26	27.10	28.39
C	Content		Std-1	Std-2	Std-3	Std-4	Std-5	Std-6
	C(t)		14.25	17.68	21.07	24.25	26.85	28.41

Figure 58. Plate spreadsheet in Quantitation Data tab.

RFU Spreadsheet

Select the **RFU** spreadsheet to see the RFU readings for each well acquired at each cycle of the experiment. Select individual fluorophores by clicking a tab at the bottom of the spreadsheet. The well number appears at the top of each column, and the cycle number appears to the left of each row (Figure 59).



Cycle	A2	A3	A4	A5	A6	A7	B2	B3	B4	B5	B6
1	6.81	2.31	1.23	2.95	-5.79	-10.6	2.05	6.76	-2.89	-14.0	-6.27
2	5.77	1.26	3.96	0.822	-6.22	-5.26	6.28	12.3	1.22	-7.55	-7.71
3	4.08	3.28	1.84	0.687	-5.05	-8.13	5.07	3.10	1.18	-4.50	-6.59
4	2.73	0.204	-1.27	-0.473	3.02	-4.26	2.70	5.57	2.42	-1.25	-2.66
5	2.69	0.220	4.31	1.42	-1.91	1.63	0.275	5.51	2.41	-1.60	-0.366

Figure 59. RFU spreadsheet in the Quantitation Data tab.

Melt Curve Tab

Open the Melt Curve tab (Figure 60) to determine the melting temperature (Tm) of amplified PCR products. This tab shows the melt curve data in these four views:

- **Melt Curve.** View the real-time data for each fluorophore as RFUs per temperature for each well
- **Melt Peak.** View the negative regression of the RFU data per temperature for each well
- **Well Selector.** Select wells to show or hide the data
- **Peak spreadsheet.** View a spreadsheet of the data collected in the selected well

  NOTE: This spreadsheet only shows as many as two peaks for each trace. To see more peaks, click the **Melt Curve Data** tab (page 70).

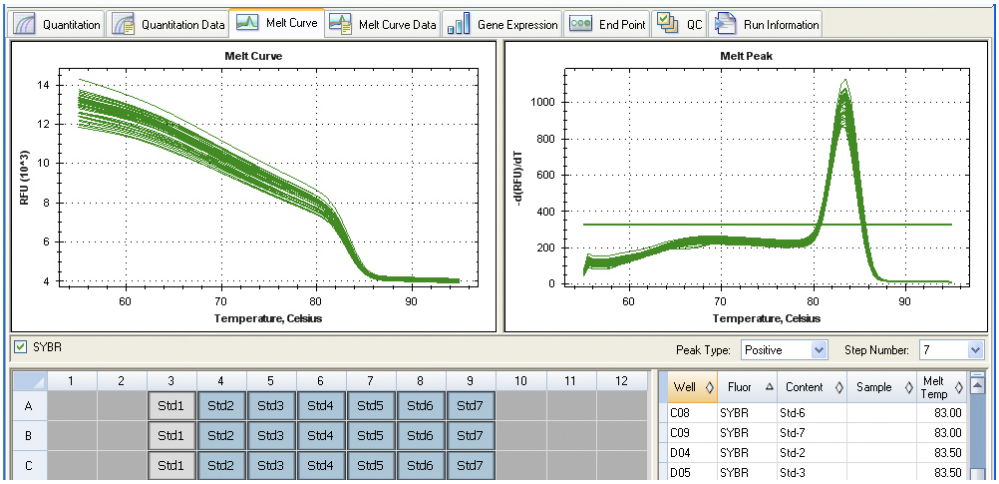


Figure 60. Layout of the Melt Curve tab in the Data Analysis window.

Adjust the Melt Curve data by any of these methods:

- Click and drag the threshold bars in the Melt Peak chart to include or exclude peaks in data analysis
- Select **Positive** in the Peak Type pull-down menu to show the spreadsheet data for the peaks above the Melt Threshold line, or select **Negative** to view the spreadsheet data for the peaks below the Melt Threshold line

Melt Curve Data Tab

The Melt Curve Data tab shows the data from the Melt Curve tab in multiple spreadsheets that include all the melt peaks for each trace. Select one of four options to show the melt curve data in different spreadsheets.

- **Melt Peaks.** List all the data, including all the melt peaks, for each trace
- **Plate.** List a view of the data and contents of each well in the plate, including the content, sample name, peak 1 and peak 2
- **RFU.** List the RFU quantities at each temperature for each well
- **–d(RFU)/dT.** List the negative rate of change in RFU as the temperature (T) changes for each well. This is a first regression plot for each well in the plate

End Point Tab

Open the End Point tab to analyze final relative fluorescence units (RFUs) for the sample wells. The software compares the RFU levels for wells with unknown samples to the RFU levels for wells with negative controls, and scores the unknown as a Positive or Negative. Positive samples have an RFU value that is greater than the average RFU value of the negative controls plus the Cut Off Value.

To analyze the end point data, the plate must contain negative controls, or the software cannot make the call. Run one of these two types of protocols:

- **Run a Quantitation protocol.** Set up a standard protocol. After running the experiment, open the Data Analysis window, adjust the data analysis settings in the Quantitation tab, and then click the End Point tab to pick an end point cycle

- **Run an End Point Only protocol.** Load the End Point Only protocol in the Plate tab of the Experiment Setup window, select or create a plate, and run the experiment

The End Point tab shows the average RFU values to determine whether or not the target was amplified by the last (end) cycle. Use these data to determine if a specific target sequence is present (positive) in a sample. Positive targets have higher RFU values than the cutoff level you define.

The software displays these data in the End Point tab:

- **Settings.** Adjust data analysis settings
- **Results.** Shows the results immediately after you adjust the Settings
- **Well Selector.** Select the wells with the end point data you want to show
- **Well spreadsheet.** Shows a spreadsheet of the end RFU collected in the selected wells

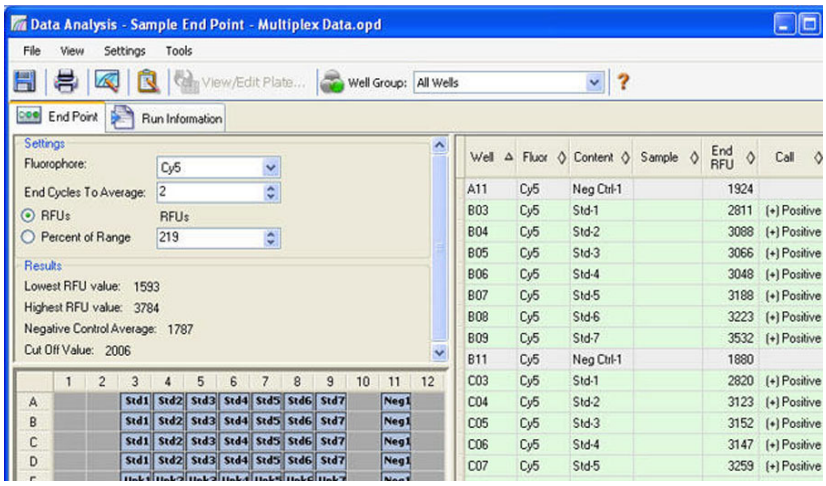


Figure 61. Layout of the End Point analysis tab.

The Results list includes this information:

- **Lowest RFU value.** Lowest RFU value in the data
- **Highest RFU value.** Highest RFU value in the data
- **Negative Control Average.** Average RFU for the wells that contain negative controls
- **Cut Off Value.** Calculated by adding the tolerance (RFU or Percentage of Range listed in the Settings) and the average of the negative controls. Samples with RFUs that are greater than the cutoff value will be called “Positive”. To adjust the cutoff value, change the RFU or Percentage of Range

The Cut Off Value is calculated using this formula:

$$\text{Cut Off Value} = \text{Negative Control Average} + \text{Tolerance}$$

Select a tolerance by one of these methods:

- **RFUs (default).** Select this method to use an absolute RFU value for the tolerance. The minimum RFU tolerance value is 2. The maximum is the absolute value of the highest RFU value minus the absolute value of the lowest RFU value. The default RFU tolerance value is 10% of the total RFU range
- **Percent of Range.** Select this method to use a percentage of the RFU range for the tolerance. The minimum percent of range is 1 percent. The maximum percent of range is 99 percent. The default percent of range is 10 percent

Adjusting the End Point Data Analysis

Adjust the information shown in the End Point tab by following these methods:

- Choose a **Fluorophore** from the pull-down list to view the data
- Choose an **End Cycle to Average** value to set the number of cycles that the software uses to calculate the average end point RFU
- Select **RFUs** to view the data in relative fluorescence units
- Select **Percentage of Range** to view the data as a percentage of the RFU range

Allelic Discrimination Tab

The Allelic Discrimination tab assigns the genotypes to wells with unknown samples using the RFU or C(t) of positive control samples (Figure 62). Use this data to identify sample genotypes, including Allele 1, Allele 2, Heterozygote, Unknown, Control 1, or Control 2.

NOTE: The data for allelic discrimination must come from multiplex experiments.

Allelic discrimination analysis requires the following minimal well contents:

- Two fluorophores in each well, except the wells that contain positive controls can contain only one fluorophore
- One fluorophore that is common to all wells in the well group
- NTC (no template control) samples if you want to normalize the data.

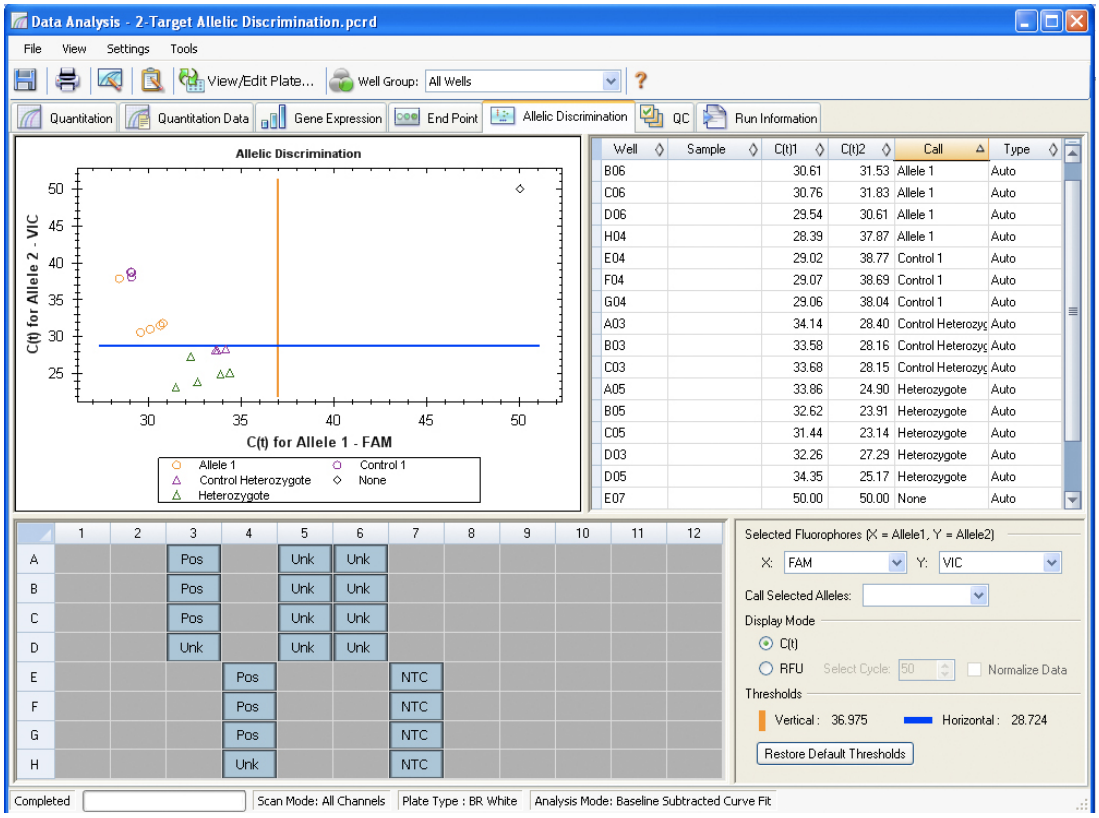


Figure 62. Layout of the Allelic Discrimination tab in the Data Analysis window.

Adjusting Data for Allelic Discrimination

The software automatically assigns a genotype to wells with unknown samples based on the positions of the vertical and horizontal threshold bars and then lists genotype calls in the spreadsheet view. To automatically call genotypes, the software uses positive controls (when available), or estimates the thresholds. The software takes an average C(t) or RFU for the positive controls to automatically set the threshold lines for discrimination of the alleles.

Adjust the positions of the threshold bars by clicking and dragging them, and the software automatically adjusts the calculations to make new genotype assignments:

- If the experiment contains three controls in the plate, then the positions of the threshold bars are based on the mean and the standard deviation of the RFU or C(t) of the controls
- If the number of controls is less than three, then the positions of the threshold bars is determined by the range of RFU or threshold cycle values in the selected fluorophore

Adjust allelic discrimination data by following any of these methods:

- Click and drag the threshold bars in the Allelic Discrimination chart to adjust the calls in the spreadsheet
- Select a fluorophore for each axis in the chart (**X:** and **Y:**) in the settings options on the bottom right of the window
- Change a call manually by highlighting a row in the spreadsheet and then selecting an option in the **Call Selected Alleles** list (including Allele 1, Allele 2, Heterozygote, None, Unknown, Control 1, or Control 2)
- Click the **Restore Default Thresholds** button to restore the vertical and horizontal bars to their original positions, which are indicated by the numbers next to the bars
- Select the **C(t) Display Mode** to view the data as threshold levels. Select **RFU Display Mode** to view the data in relative fluorescence units at the selected cycle
- Select **Normalize Data** to normalize the RFU data shown in the chart and spreadsheet

Normalization changes the data on the chart to a range from 0 to 1 on both axes. To normalize the data, the plate must contain wells with no template control (NTC) sample types for both Allele 1 and Allele 2. For this plot, the RFU data are normalized to the NTC values as a linear combination of Allele 1- and Allele 2-specific RFUs. This plot is an effective way to present RFU data.

The calculations for normalized RFUs follow the formulas presented in Livak et al. (1995).

$$\text{Normalized } A_1 = \frac{A_1}{A_1 + A_2 + \bar{x}(NTC_{A1 + A2})}$$

Where:

- A_1 represents RFU for Allele 1
- A_2 represents RFU for Allele 2
- $\bar{x}$ represents the mean RFU

$NTC_{A1 + A2}$ represents the sum of RFUs for the NTC sample of Allele 1 and Allele 2

QC Tab

Open the **QC tab** to quickly assess the quality of the experimental data based on the rules defined in the QC tab in the User Preferences window (see “QC Tab” on page 96).

The software displays the currently applied QC rules and the settings that define each rule (Figure 63). The rule description also displays wells that fail a selected rule.

NOTE: You can turn on or turn off rules by clicking the check box next to the rule in the Use Rule column.

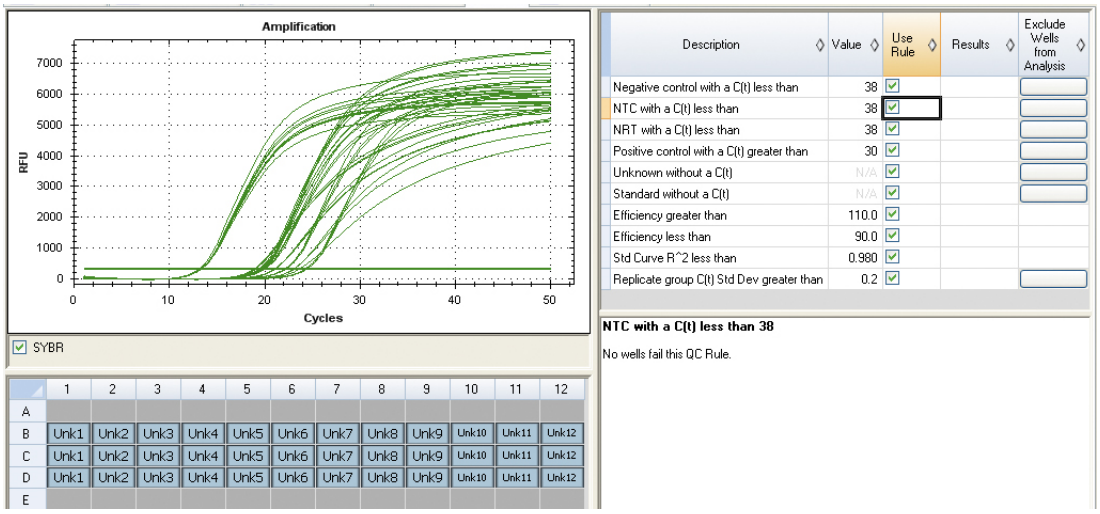


Figure 63. QC tab layout.

Run Information Tab

The Run Information tab (Figure 64) shows the protocol and other information about the run for each experiment. You can also enter and edit the run Notes by typing in the Notes box or enter and edit the data ID for the run by typing in the ID box.

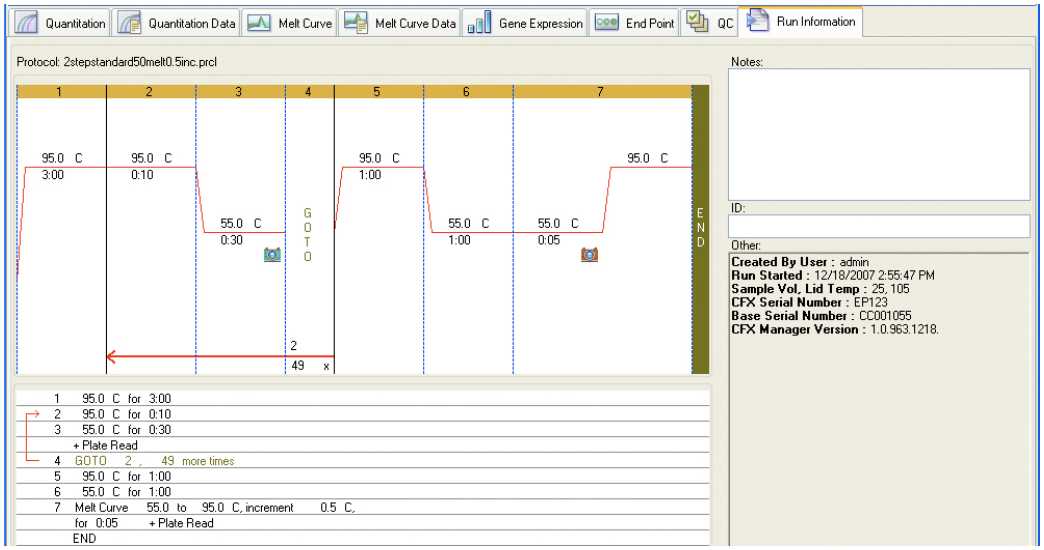


Figure 64. Layout of the Run Information tab in the Data Analysis window.

Data File Reports

The Report window (Figure 65) shows information about the current data file in the Data Analysis window. To open a report, select **Tools > Reports**, or click the **Reports** button on the toolbar in the Data Analysis window.

The Report window shows these three sections:

- **Menu and toolbar.** Select options to format, save, and print the report or template
- **Options list (top, left side of window).** Select options to show in the report
- **Options pane (bottom, left side of window).** Enter information about a selected option
- **Preview pane (right side of window).** View the current report in a preview

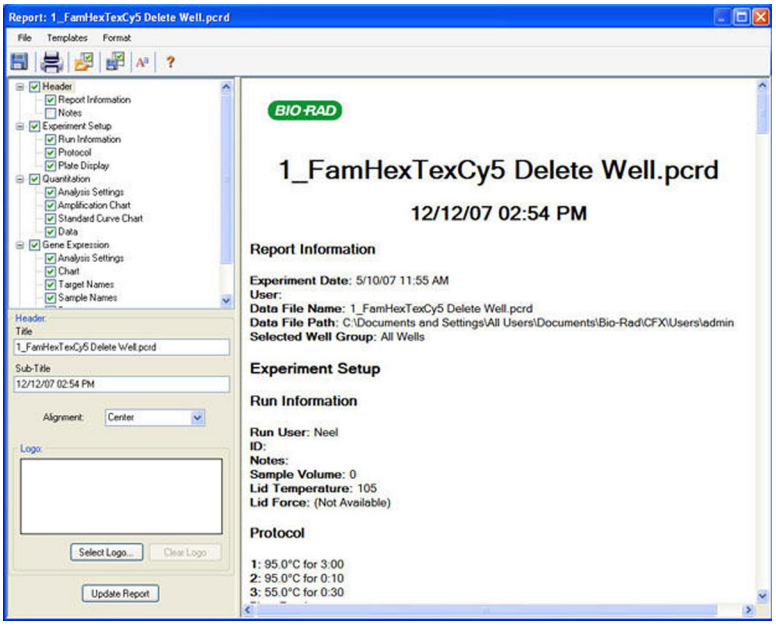


Figure 65. Example of a Report window for a data file.

TIP: The layout of the report can define the type of information that appears in any report if you save the report as a template. Select **Template > Save** or **Save As** to save the layout of the current report as a template.

Create a Data Analysis Report

To create a report in the Data Analysis window, follow these steps:

1. Make final adjustments to the well contents, selected wells, charts, and spreadsheets in the Data Analysis window before creating the report.
2. Click the **Report** button in the Data Analysis toolbar to open the Report window.
3. Change the options you want to include in the report. The report opens with default options selected. Click the check boxes in the report options list to change whole categories or individual options within a category.

   NOTE: The data that appear in the report are dependent on the current selections within the tabs of the Data Analysis window. For example, a quantitation experiment might not contain a standard curve, and therefore those data do not appear in the Data Analysis window or in the data report.

4. Click the **Update Report** button to update the Report Preview with any changes.
5. Print or save the report. Click the **Print** button in the toolbar to print the current report. Select **File > Save** to save the report as a PDF (Adobe Acrobat Reader file), MHT (Microsoft document), or MHTML (Microsoft document) formatted file, and select a location to store the file. Select **File > Save As** to save the report with a new name or in a new location.
6. (Optional) Create a report template with the information you want. To save the current report settings in a template, select **Template > Save** or **Save As**. Then load the report template the next time you want to make a new report.

Data Analysis Report Categories

A report can include any of the options in each category described in Table 22, depending on the type of data in the Data Analysis window.

Table 22. Data analysis report categories in the options list.

Category	Option	Description
Header		Title, subtitle and logo for the report
	Report Information	Experiment date, user name, data file name, data file path, and selected well group
	Notes	Notes about the data report
Experiment Setup		
	Run Information	Includes the experiment date, user, data file name, data file path, and the selected well group
	Protocol	Text view of the protocol steps and options
	Plate Display	Show a plate view of the information in each well of the plate
Quantitation		
	Analysis Settings	Includes the step number when data were collected, the analysis mode, and the baseline subtraction method
	Amplification Chart	Copy of the amplification chart for experiments that include quantitation data
	Standard Curve Chart	Copy of the standard curve chart
	Data	Spreadsheet listing the data in each well
Gene Expression		
	Analysis Settings	Includes the analysis mode, chart data, scaling option, and chart error
	Chart	Copy of the gene expression chart
	Target Names	Chart of the names
	Sample Names	Chart of the names
	Data	Spreadsheet listing the data in each well
Melt Curve		

Table 22. Data analysis report categories in the options list. (continued)

Category	Option	Description
	Analysis Settings	Includes the melt step number and threshold bar setting
	Melt Curve Chart	Copy of the melt curve chart
	Melt Peak Chart	Copy of the melt peak chart
	Data	Spreadsheet listing the data in each well
Allelic Discrimination		
	Analysis Settings	Includes display mode, fluorophores, cycle, thresholds, and normalized data
	Chart	Copy of the allelic discrimination chart
	Data	Spreadsheet listing the data in each well
End Point		
	Analysis Settings	Includes fluorophore, end cycles to average, mode, lowest RFU value, highest RFU value, and cutoff value
	Data	Spreadsheet listing the data in each well

9 Gene Expression Analysis

Read this chapter for information about performing Gene Expression Analysis:

- Gene Expression (page 79)
- Plate setup for gene expression analysis (page 80)
- Gene Expression tab (page 80)
- Experiment Settings window (page 85)
- Gene Study (page 86)
- Gene Study Data spreadsheet (page 89)
- Gene Study Report window (page 90)

Gene Expression

With the use of stringently qualified controls in your reactions, you can run a gene expression experiment to normalize the relative differences in a target concentration between samples. Typically, message levels for one or more reference genes are used to normalize the expression levels of a gene of interest. Reference genes take into account loading differences or other variations represented in each sample, and they should not be regulated in the biological system being studied.

Open the Gene Expression tab to evaluate relative differences between PCR reactions in two or more wells. For example, you can evaluate relative numbers of viral genomes, or relative numbers of transfected sequences in a PCR reaction. The most common application for gene expression study is the comparison of cDNA concentration in more than one reaction to estimate the levels of steady state messenger RNA.

The software calculates the relative expression level of a target with one of these scenarios:

- Relative expression level of a target sequence (Target 1) relative to another target (Target 2). For example, the amount of one gene relative to another gene under the same sample treatment
- Relative expression level of one target sequence in one sample compared to the same target under different sample treatments. For example, the relative amount of one gene relative to itself under different temporal, geographical, or developmental conditions

Plate Setup for Gene Expression Analysis

To perform gene expression analysis, the contents of the wells must include the following:

- **Two or more targets.** The two targets that represent different amplified genes or sequences in your samples
- **One or more reference targets.** At least one target must be a reference target for normalized expression. Assign all reference targets in the Experiment Settings window (page 38) to analyze the data in Normalized Expression mode (ΔΔC(t)). Experiments that do not contain a reference must be analyzed using Relative Expression mode (ΔC(t))
- **Common samples.** Your reactions must include common samples (minimum of two required) to view your data plotted in the Gene Expression tab. These samples represent different treatments or conditions for each of your target sequences. Assign a control sample (optional) in the Experiment Settings window (page 38)

The requirements for Gene Expression setup in the Plate Editor depend on whether reaction contents are **singleplex PCR** with one fluorophore in the reactions or **multiplex PCR** with more than one fluorophore in the reactions.

Figure 66 shows an example of the minimum contents of the wells for a singleplex gene expression experiment.

Unk	Unk
Target1 Sample1	Target1 Sample2
Unk	**Unk**
Target2 Sample1	Target2 Sample2

Figure 66. Example of well contents in a singleplex gene expression experiment.

Figure 67 shows an example of the minimum contents of the wells for a multiplex gene expression experiment.

Unk	Unk
Target1 Target2 Sample1	Target1 Target2 Sample2

Figure 67. Example of well contents in a multiplex gene expression experiment.

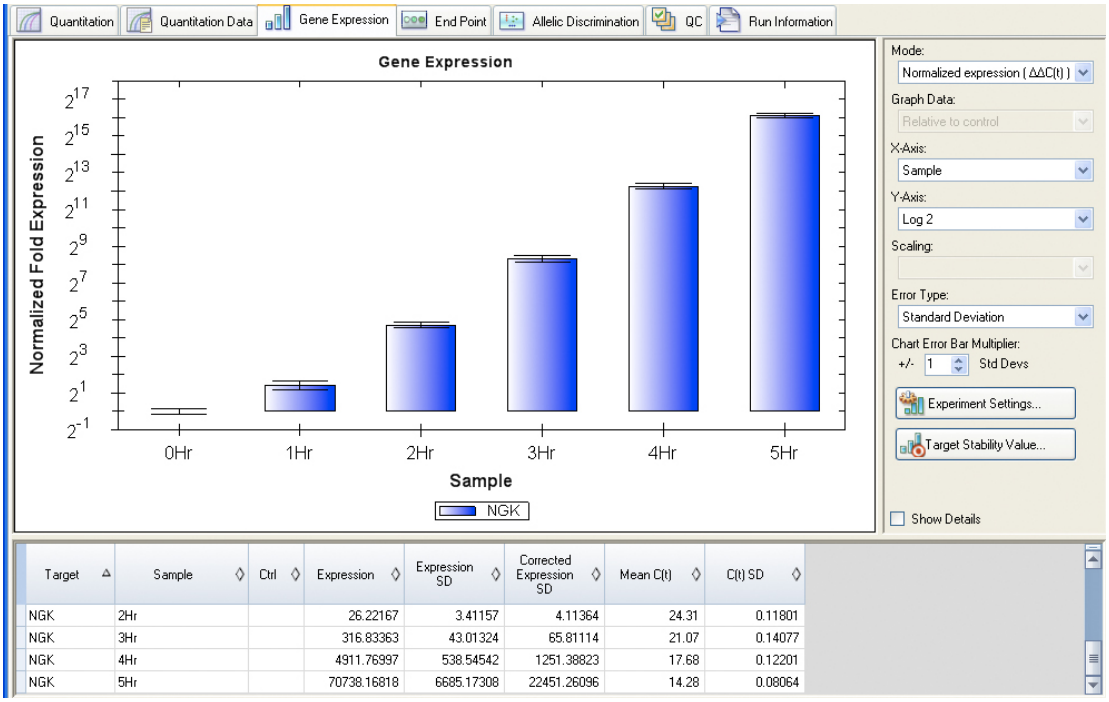
Gene Expression Tab

The Gene Expression tab in the Data Analysis window shows the relative expression of targets in these two views:

- **Gene Expression chart.** Shows the real-time PCR data as normalized expression (ΔΔC(t)) or relative quantity (ΔC(t))
- **Spreadsheet.** Shows a spreadsheet of the gene expression data

  TIP: Right-click any chart or spreadsheet for options. Click the **View/Edit Plate** button to open the Plate Editor, and change well contents in the plate.

.



Target	Sample	Ctrl	Expression	Expression SD	Corrected Expression SD	Mean C(t)	C(t) SD
NGK	2Hr		26.22167	3.41157	4.11364	24.31	0.11801
NGK	3Hr		316.83363	43.01324	65.81114	21.07	0.14077
NGK	4Hr		4911.76997	538.54542	1251.38823	17.68	0.12201
NGK	5Hr		70738.16818	6685.17308	22451.26096	14.28	0.08064

Figure 68. Layout of the Gene Expression tab in the Data Analysis window.

TIP: Right-click on the chart to select right-click menu options. Select **Sort** from this menu to rearrange the order of the Target and Sample names in the chart.

Normalized Gene Expression

To normalize data, use the measured expression level of one or more reference genes (targets) as a normalization factor. Reference genes are targets that are not regulated in the biological system being studied, such as actin, GAPDH, or Histone H3.

To set up normalized gene expression (ΔΔC(t)) analysis, follow these steps:

1. Open a data file (.pcrd extension).
2. Review the data in the Quantitation tab of the Data Analysis window. Make adjustments to the data, such as changing the threshold and the Analysis Mode.
3. Click the **Gene Expression** tab.
4. Choose a control in the **Samples** tab of the Experiment Settings window. If a control is assigned, the software normalizes the relative quantities for all genes to the control quantity, which is set to 1.
5. Select reference genes for this experiment in the Target tab of the Experiment Settings window. Gene expression analysis requires one reference among the targets in your samples.
6. Select **Normalized Expression (ΔΔC(t))** if it is not already selected, and then view the expression levels in the Gene Expression tab.

Relative Quantity

Select **Relative Quantity (ΔC(t))** from the pull-down menu in the chart controls of the Gene Expression tab to run a Relative Quantity (ΔC(t)) analysis. By definition, relative quantity (ΔC(t)) data are not normalized. This method is used to quantitate samples that do not include any reference genes (targets). Typically, the researcher is confident in one of the following considerations when setting up the experiment:

- Each sample represents the same amount of template in each biological sample, possibly the same mass of RNA or cDNA in each well
- Any variance in the amount of biological sample loaded will be normalized after the run by some method in the data analysis outside of the software. For example, a researcher might choose to divide the relative quantity value by the normalizing factor, possibly the mass of nucleic acid loaded for each sample or the number of cells from which the nucleic acid was isolated.

Adjusting Gene Expression Data

After selecting your analysis method, adjust the data you view in the Gene Expression tab by changing the settings options to the right of the chart.

Graph Data

Graph data options allow you to present the data in the graph with one of two options:

- **Relative to control.** Graph the data with the axis scaled from 0 to 1. If you assign a control in your experiment, select this option to quickly visualize upregulation and downregulation of the target
- **Relative to zero.** Graph the data with the origin at zero

X-Axis Options

The x-axis option allows you to select the x-axis data of the Gene Expression graph:

- **Target.** Select this option to graph the target names on the x-axis
- **Sample.** Select this option to graph the sample names on the x-axis

Y-Axis Options

The y-axis option allows you to show the Gene Expression graph in one of three scales:

- **Linear.** Select this option to show a linear scale
- **Log 2.** Select this option to evaluate samples across a large dynamic range
- **Log 10.** Select this option to evaluate samples across a very large dynamic range

Scaling Options

Select **Normalized Gene Expression (ΔΔC(t))** to activate the scaling options in the Gene Expression graph. Select one of these scaling options to calculate and present your data in a manner that best suits your experimental design:

- **Unscaled expression.** This option presents the unscaled normalized gene expression
- **Highest expression.** Scale the normalized gene expression to the highest for each target by dividing the expression level of each sample by the highest level of expression in all the samples. This scaling option uses the scaled to highest formula

- **Lowest expression.** Recalculate the normalized gene expression for each target by dividing the expression level of each sample by the lowest level of expression in all the samples. This scaling options uses the scaled to lowest formula

Error Type

Select an option for the type of error calculations (error bars) in the Gene Expression graph:

- Standard Error of the Mean (default, SEMs)
- Standard Deviation (Std Devs)

Chart Error Bar Multiplier

Select a multiplier for the error bars in the Gene Expression graph. Select one of these integers: 1 (default), 2, or 3. The type of multiplier changes when you select the Error Type:

- SEMs for Standard Error of the Mean
- Std Devs for Standard Deviations

Target Stability Value

Open this window whenever more than 1 reference gene is used. The software calculates two quality parameters for the reference genes:

- **Coefficient of Variation (CV)** of normalized reference gene relative quantities. Lower CV values denotes higher stability
- **M-value**. A measure of the reference gene expression stability

Right-Click Menu Options for Gene Expression Graph

Right-click on the Gene Expression graph to select the items shown in Table 23.

Table 23. Right-click menu items.

Item	Function
Copy	Copy the chart to a clipboard
Save as Image	Save the graph in the chart view as an image file. The default image type is PNG. The other selections for image file types include GIF, JPG, TIF, and BMP
Page Setup...	Select a page setup for printing
Print...	Print the chart view
Show Point Values	Display the relative quantity of each point on the graph when you place the cursor over that point
Set Scale to Default	Set the chart view back to the default settings after magnifying it
Chart Options...	Open the Chart Options window to adjust the graph
Sort	Sort the order that samples or targets appear on the chart x-axis
User Corrected Std Devs	Calculate the error bars using the corrected standard deviation formula
Use Solid Bar Colors	Display solid bars in the graph
x-axis labels	Choose to display x-axis labels horizontal or angled

Gene Expression Spreadsheet

Table 24 describes the information shown in the Gene Expression spreadsheet.

Table 24. Description of information in the spreadsheet on the Gene Expression tab.

Information	Description
Target	Target Name (amplified gene) selected in the Experiment Settings window
Sample	Sample Name selected in the Experiment Settings window
Ctrl	Control sample, when the Sample Name is selected as a control in the Experiment Settings window
Expression	Normalized Gene Expression ($\Delta\Delta$C(t)) or Relative quantity (ΔC(t)) depending on the selected mode
Expression SEM (or SD)	Standard Error of the Mean or Standard Deviation, depending on the selected option
Corrected Expression SEM (or SD)	Corrected value calculation for Standard Error of the Mean (SEM) or Standard Deviation (SD) of the relative expression, depending on the selected option
Mean (C(t))	Mean of the threshold cycle
C(t) SEM (or SD)	Standard Error of the Mean or Standard Deviation of the threshold cycle, depending on the selected option

Show Details Option

When you click the Show Details check box, Table 25 also shows this information.

Table 25. Information in Gene Expression spreadsheet with Show Details selected.

Information	Description
Data Set	Fluorescence data from one fluorophore in the data file
Relative Quantity	Calculated relative quantity of samples
Relative Quantity SD	Standard deviation of the relative quantity calculation
Corrected Relative Quantity SD	Calculated standard deviation of the corrected relative quantity
Unscaled Expression	Calculated unscaled expression
Unscaled Expression SD	Calculated standard deviation of the unscaled expression
Corrected Unscaled Expression SD	Corrected standard deviation of the unscaled expression
Expression	Relative expression level
Wells	Well number in the plate

Experiment Settings Window

Open the Experiment Settings window by clicking the **Experiment Settings** button in the Gene Expression tab. In this window, view or change the list of Targets and Samples, select reference genes, select control samples, or set the Gene Expression Analysis sample group to be analyzed if Collection Names have been added to the wells (Figure 69).

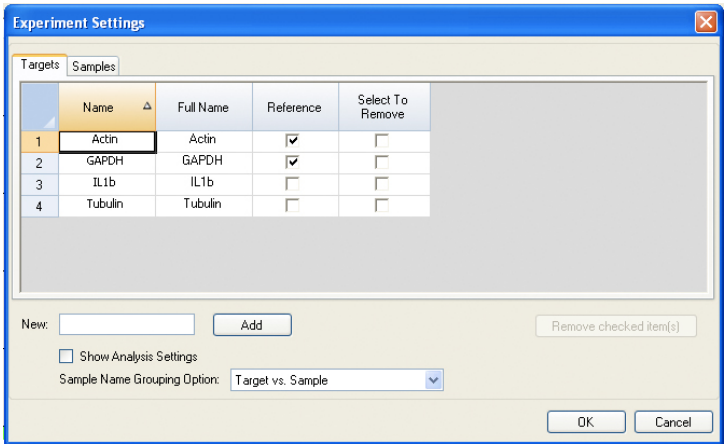


Figure 69. Experiment Settings window with Targets tab selected.

To adjust the lists in these tabs, use the following functions:

- Add a target or sample name by typing a name in the **New** box, and clicking **Add**
- Remove a target or sample name from the list by clicking the **Select to Remove Name** box for that row, and then clicking the **Remove checked item(s)** button
- Select the target as a reference for gene expression data analysis by clicking the box in the **Reference** column next to the **Name** for that target
- Select the sample as a control sample for gene expression data analysis by clicking the box in the **Control** column next to the name for that sample

Sample Name Grouping Option

Loading **Collection Names** in the wells enables samples to be analyzed in one of four configurations defined by the Sample Name Grouping Option. These options are available from the pull-down menu in the Experiment Settings tab.

- **Target vs. Sample.** Only the well sample name is used in the gene expression calculations
- **Target vs. Collection.** Only the well collection name is used in the calculations
- **Target vs. Sample_Collection.** The sample name and collection name are combined to make a single name that is used in the calculations
- **Target vs. Collection_Sample.** The collection name and sample name are combined to make a single name that is used in the calculations

Show Analysis Settings in Experiment Settings

Click the **Show Analysis Settings** box in the Experiment Settings window to view or change analysis parameters applied in the Gene Expression tab:

- Click a cell in the **Color** column to change the color of the targets graphed in the Gene Expression chart

- Enter a number for the efficiency of a target. The software will calculate the relative efficiency for a target using **Auto Efficiency** if the data for a target includes a standard curve. Alternatively, type a previously determined efficiency

Figure 70 shows the efficiency of all the targets, which appears if **Auto Efficiency** is selected.

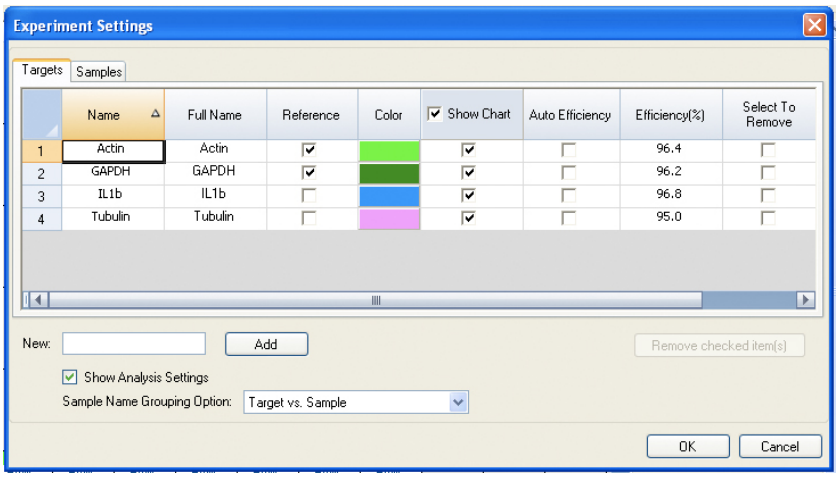


Figure 70. Targets tab in Experiment Settings window with Analysis Settings selected.

To adjust the settings for a sample in the Samples tab:

- Click a color in the **Color** column to change the color of the samples graphed in the Gene Expression chart
- Click a box in the **Show Chart** column to show the sample in the Gene Expression chart using a color that is selected in the Color column

Gene Study

Create a Gene Study to compare gene expression data from one or more real-time PCR experiments using an inter-run calibrator to normalize data between the experiments. Create a Gene Study by adding data from one or more data files (.pcrd extension) to the Gene Study; the software groups them into a single file (.mgxd extension).

NOTE: The gene expression data must include a common sample in every data file to create a Gene Study. The software uses the common sample to normalize the data between experiments. Select the sample names in the Experiment Settings window (page 38).

NOTE: The maximum number of samples you can analyze in a Gene Study is limited by the size of the computer's RAM and virtual memory.

Gene Study Inter-Run Calibration

All data within the Gene Study are normalized by inter-run calibrator to calculate the smallest average ΔC(t) value. When the data files within the Gene Study include more than one inter-run calibrator, then the calibrator with the smallest average ΔC(t) value becomes the dominant inter-run calibrator. The dominant calibrator is used to adjust all C(t) values in the Gene Study.

To find the dominant inter-run calibrator, the software calculates the average of the ΔC(t) values for all inter-run calibrators of a given target (gene), and then uses a multitiered algorithm to determine the dominant inter-run calibrator within all the data. The algorithm for finding the dominant inter-run calibrator includes the following hierarchy:

1. Set the dominant calibrator to the target with the highest number of common replicate groups in a given pair-wise comparison.
2. If any target has the same number of common replicate groups, then set the dominant calibrator to the target with the smallest range of ΔC(t) values in pair-wise comparisons. The range is examined by comparing the absolute value of the difference between the maximum and minimum ΔC(t) for the inter-run calibrators of a given target.
3. If any target has an identical range as the ΔC(t) values, then set the dominant calibrator to the target with the smallest absolute value of average ΔC(t) for eligible inter-run calibrator samples.
4. If any target has identical average ΔC(t) absolute values, then set the dominant calibrator to the replicate group with the smallest ΔC(t).

   NOTE: The first data file imported into the Gene Study will always serve as the hub file for pairwise data comparison during inter-run calibration.

Gene Study Window

The Gene Study window includes two tabs:

- **Study Setup tab.** Click this tab to manage the experiments in the Gene Study. Adding or removing data files in a Gene Study does not change the original data in that file
- **Study Analysis tab.** Click this tab to view the gene expression data for the combined experiments

Figure 71 shows the Gene Study window.

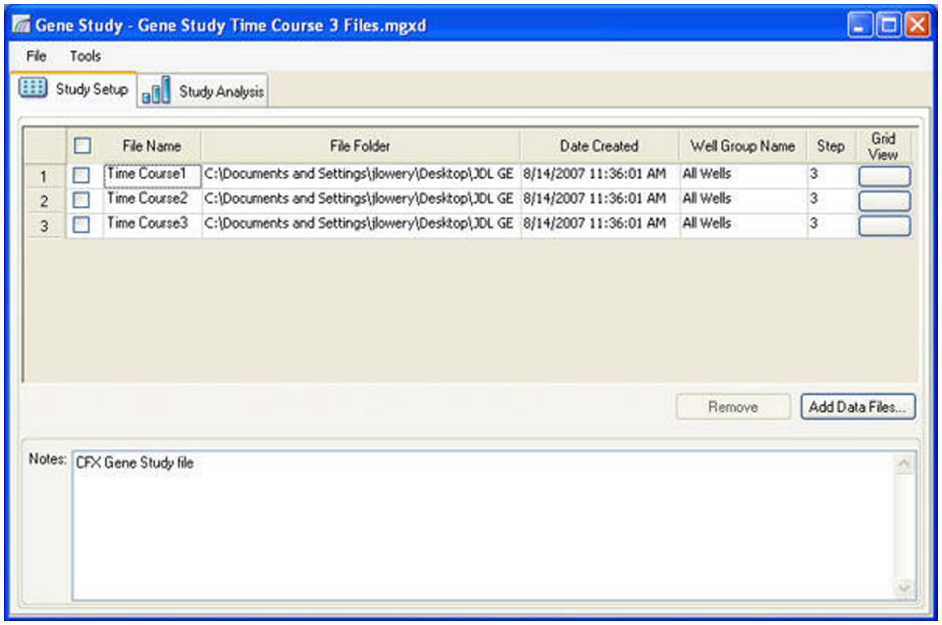


Figure 71. Gene Study window.

Study Setup Tab

Before importing data into a Gene Study, do the following in the Data Analysis window:

- Check that samples that contain the same content are named with the same name. In a Gene Study, the software assumes that wells with the same Target or Sample name contain the same samples
- Adjust the baseline and threshold (C(t)) in the Quantitation tab to optimize the data in each experiment before you add them to a Gene Study
- Select the well group you want to include in the Gene Study

The Study Setup tab (Figure 71) shows a list of all the experiments in the Gene Study.

- **Add experiments.** Click the **Add Data Files** button to select a file from a browser window. To quickly add experiments to a Gene Study, drag the data files (.pcrd extension) to the Gene Study window
- **Remove experiments from this Gene Study.** Select one or more files in the list and click **Remove**
- **Add notes about the Gene Study.** Type in the Notes box to add comments about the files and analysis in this Gene Study

The Study Setup tab lists the data files in the Gene Study, as described in Table 26.

Table 26. Study Setup tab in Gene Study window.

Column Title	Description
File Name	Name of the experiment data file (.pcrd extension)
File Folder	Directory that stores the data file for each experiment in the Gene Study
Date Created	Date the run data were collected
Well Group Name	Name of the well group that was selected when the file was added to the Gene Study TIP: To analyze one well group in the Gene Study, that well group must be selected in the Data Analysis window before importing the data file into the Gene Study
Step	Protocol step that included the plate read to collect real-time PCR data
Grid View	Open a plate map of the plate with the data in each of the experiments included in the Gene Study

Study Analysis Tab

The Study Analysis tab shows the data from all experiments that are added to the Gene Study. Open this tab to analyze the data, and select these options for the Gene Expression chart:

- **Mode.** Select **Normalized Expression (ΔΔC(t))** or **Relative Quantity (ΔC(t))**
- **Graph Data.** Select **Relative to normal** or **Relative to control** in the graph
- **x-axis options.** Select the labels on the x-axis of the graph, including Sample or Target
- **y-axis options.** Change the labels on the y-axis of the graph, including Linear, Log 2, or Log 10
- **Scaling Options.** Choose **Highest** value, **Lowest** value, or leave the data **Unscaled**. This option is only available when your samples do not contain controls

- **Graph Error.** Select the multiplier for standard deviation bars in the graph, including ±1, 2, or 3
- **Experiment Settings button.** Choose the show options for targets and samples in the Experiment Settings window
- **Show Details check box.** Click **Show Details** to add more columns of data to the chart

Highlighting a sample in the Gene Expression chart highlights the corresponding cell in the spreadsheet below the chart (Figure 72).

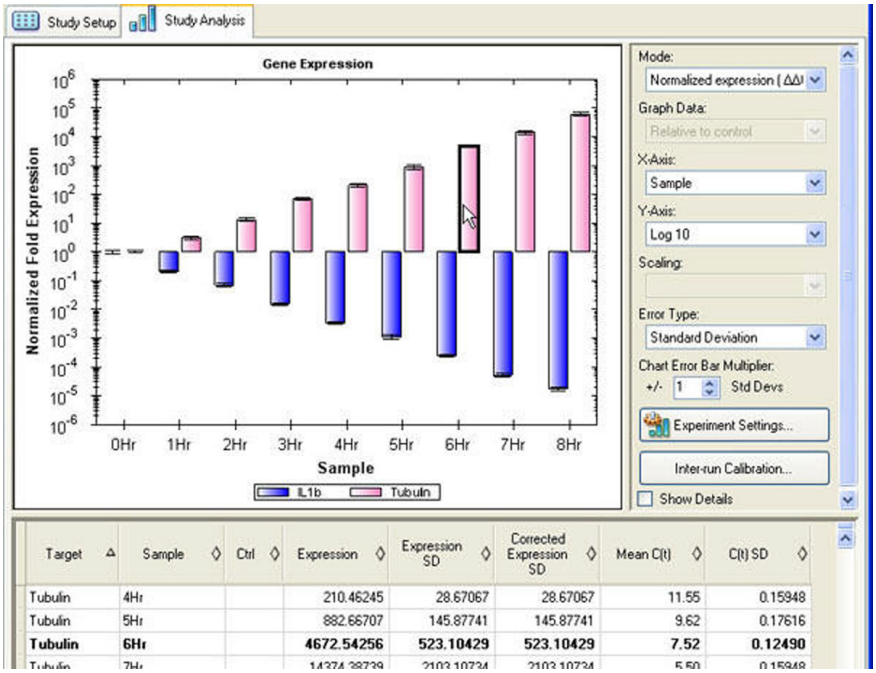


Figure 72. Study Analysis tab in Gene Study window.

Gene Study Data Spreadsheet

The data spreadsheet in the Gene Study window lists information about each target and sample in the Gene Study (Figure 72).

Table 27 describes the information shown in the Gene Study spreadsheet.

Table 27. Information in the spreadsheet on the Study Analysis tab.

Information	Description
Target	Target Name (amplified gene) selected in the Experiment Settings window
Sample	Sample Name selected in the Experiment Settings window
Ctrl	Control sample, when the sample name is selected as a control in the Experiment Settings window
Expression	Normalized Gene Expression (ΔΔC(t)) or Relative Quantity (ΔC(t)), depending on the selected mode
Expression SEM (or SD)	Standard Error of the Mean or Standard Deviation, depending on the selected option

Table 27. Information in the spreadsheet on the Study Analysis tab. (continued)

Information	Description
Corrected Expression SEM (or SD)	Corrected value calculation for Standard Error of the Mean (SEM) or Standard Deviation (SD) of the relative expression, depending on the selected option
Mean (C(t))	Mean of the threshold cycle
C(t) SEM (or SD)	Standard Error of the Mean or Standard Deviation of the threshold cycle, depending on the selected option

Show Details Data

Click the Show Details check box to show additional information. The spreadsheet adds the information in the columns listed in Table 28.

Table 28. Information added to the spreadsheet when Show Details selected.

Information	Description
Data Set	Fluorescence data from one fluorophore in one data file
Relative Quantity	Calculated relative quantity of samples
Relative Quantity SD	Standard deviation of the relative quantity calculation
Corrected Relative Quantity SD	Calculated standard deviation of the corrected relative quantity
Unscaled Expression	Calculated unscaled expression
Unscaled Expression SD	Calculated standard deviation of the unscaled expression
Corrected Unscaled Expression SD	Corrected standard deviation of the unscaled expression
Expression	Relative expression
Wells	Well number in the plate

Gene Study Report Window

Open the Gene Study Report window to arrange the Gene Study data into a report. To create a gene study report, follow these steps:

1. Adjust the Gene Study report data and charts as needed before creating a report.
2. Select **Tools > Reports** to open the Gene Study report window.
3. Click the check boxes in the report options list to select and remove options to choose the data to display.
4. Click the **Update Report** button to update the report preview pane.The report preview pane shows a view of the report.
5. Print or save the report. Click the **Print** button in the toolbar to print the current report. Select **File > Save** to save the report as a PDF (Adobe Acrobat Reader file), MHT (Microsoft document), or MHTML (Microsoft document) formatted file, and select a location to store the file. Select **File > Save As** to save the report with a new name or in a new location.

10 Users and Preferences

Read this chapter to learn more about managing software users and their preferences:

- Log In or Select User (page 91)
- User Preferences window (page 92)
- Configure email notification (page 93)
- User Administration (page 97)

Log In or Select User

CFX Manager™ software manages multiple users and their preferences. The current, logged in software user is displayed at the top of the main software window (Figure 73).

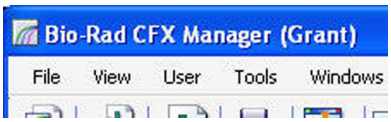


Figure 73. User name displayed.

CFX Manager software manages who logs in to the software through the Login dialog box (Figure 74). When you start the software, the Login dialog box opens automatically if there are two or more users listed in the User Administration window.

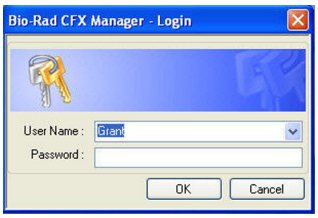


Figure 74. Login dialog box.

Log in to the software, or switch users by following these steps:

1. Open the Login dialog box, if it is not already open, by clicking the **Select User** button in the toolbar or selecting **User > Select User** in the menu bar.
2. Select a name from the **User Name** pull-down list. The default is “Admin” (administrator).
3. Type a password in the **Password** box.
4. Click **OK** to close the Login dialog box and open the software.

5. To add a new user name and password, contact your software administrator.

Change a Password

Change a password by following these steps:

1. Select **User > Change Password** from the main software window menu to open the Change Password dialog box.
2. Enter the old password in the Old Password box.
3. Enter the new password in the New Password and the Confirm New Password boxes.
4. Click **OK** to confirm the change.

User Preferences Window

CFX Manager software tracks the preferences of each user that logs in to the software. To change user preferences, open the User Preferences window using one of these methods:

- Click the **User Preferences** button in the main software window toolbar
- Select **User > User Preferences** in the main software window menu bar
- Click one of the tabs (Figure 75) to view or change preferences

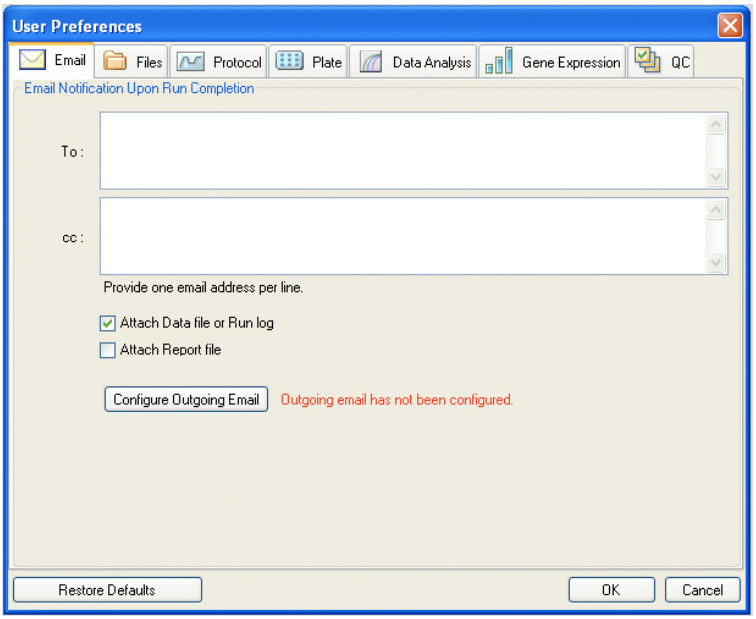


Figure 75. User Preferences window with tabs.

Email Tab

Select the **Email** tab (Figure 75) to enter the email addresses where you want to receive confirmation of the completion of the run. The software can send an attached data file or report file with the email when the check boxes next to these options are checked.

Configure Email Notification

Click the **Configure Outgoing Email** button to open the Options window (Figure 76) to configure the SMTP server and send a test email from the computer. Input the following:

- **SMTP Server Name.** The name of the SMTP server as provided by your ISP
- **Port.** The port number of your SMTP server, as provided by your ISP; this is usually 25
- **Use SSL.** Whether to use Secure Sockets Layer. Some SMTP servers require this to be used, others require that it not be used
- **Use Default "From" Address.** This can usually be left in the default checked state. However, some SMTP servers require all sent email to have a "from" address that is from a certain domain, i.e.<name>@YourCompany.com. If that is the case, this checkbox must be unchecked, and a valid "from" email address must be supplied in the box labeled "From" Address:
- **Authentication Required.** Many SMTP servers require authentication. If so, this checkbox must be checked, and a User Name and Password must be supplied
- **Test email.** To test the email settings, enter one or more email addresses in **Test Email Address** text box. Multiple email addresses can be separated by a comma. Then click the **Test Email** button

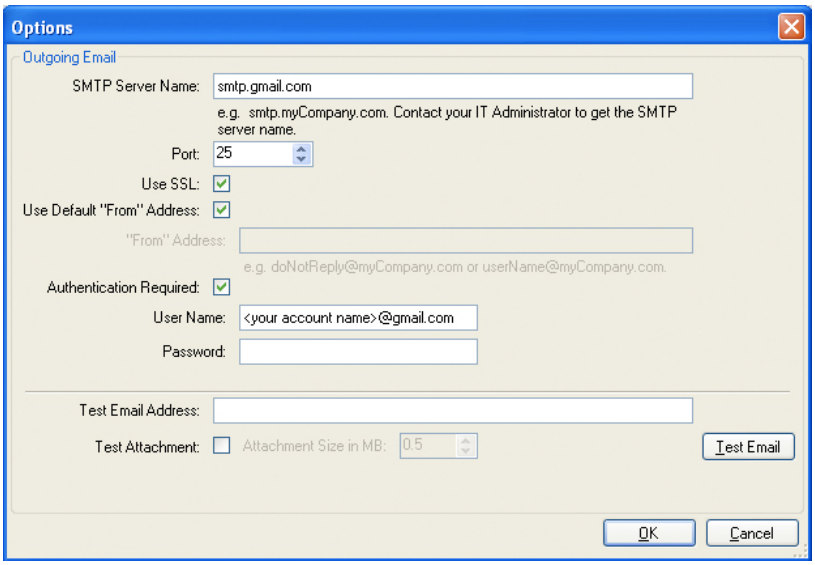


Figure 76. Options to configure email.

NOTE: Some SMTP servers do not allow attachments, and others allow attachments only up to certain sizes. If you will use CFX Manager software to email Data Files and/or Reports, you may want to test your server's ability to email attachments by checking the Test Attachment box, and setting the Attachment Size in MB with up to 5 megabytes (MB) or more.

Files Tab

Select the **Files** tab to list the default locations for opening and saving files. Click the "..." button to the right of each box to open a browser window and locate a folder

- **Default Folder for File Creation.** Select a default folder where you want to save new files. Select a location for each file type (Protocol, Plate, Data, or Gene Study file)

- **File Selection for Experiment Setup.** Select the default protocol and plate files that appear when you open the Experiment Setup window
- **Data File Prefix.** Define the beginning text of the file name for data files.

Protocol Tab

Select the **Protocol** tab in the User Preferences window to specify the default settings for a new protocol file in the Protocol Editor window:

- **Protocol Editor.** Set the default settings that appear in the Protocol Editor. Select a default sample volume to describe the volume of each sample in the wells and select a lid shutoff temperature
- **Protocol AutoWriter.** Selects default settings that appear in the Protocol AutoWriter

Plate Tab

Select the **Plate** tab in the User Preferences window (Figure 77) to specify the following default settings for a new Plate file in the Plate Editor window:

- **Plate Type.** Select the default plate type
- **Plate Size.** Select the default plate size
- **Units.** Select the units used to describe the concentration of the starting template for wells that contain standards.
- **Scientific Notation.** Select scientific notation to view concentration units in that notation
- **Scan Mode.** Select a default scan mode
- **Fluorophores.** Click check boxes to select the default fluorophores that appear in the Plate Editor well loading controls
- **Libraries.** Enter the target and sample names used in your experiments. These names appear in the lists in the Targets tab and Samples tab in the Experiment Settings window

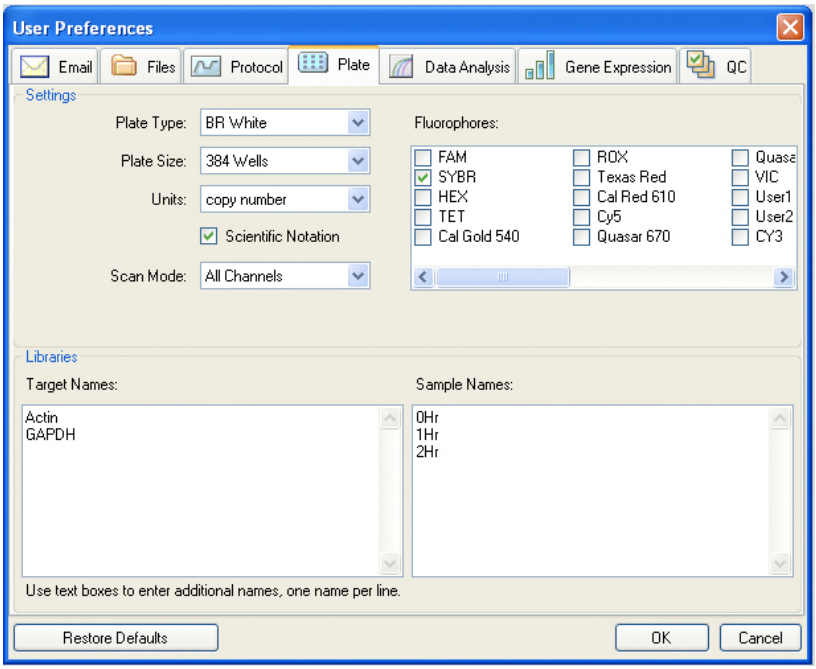


Figure 77. Plate tab in the User Preferences window.

Data Analysis Tab

Select the **Data Analysis** Tab in the User Preferences window to change the default settings for data that appear in the Data Analysis window.

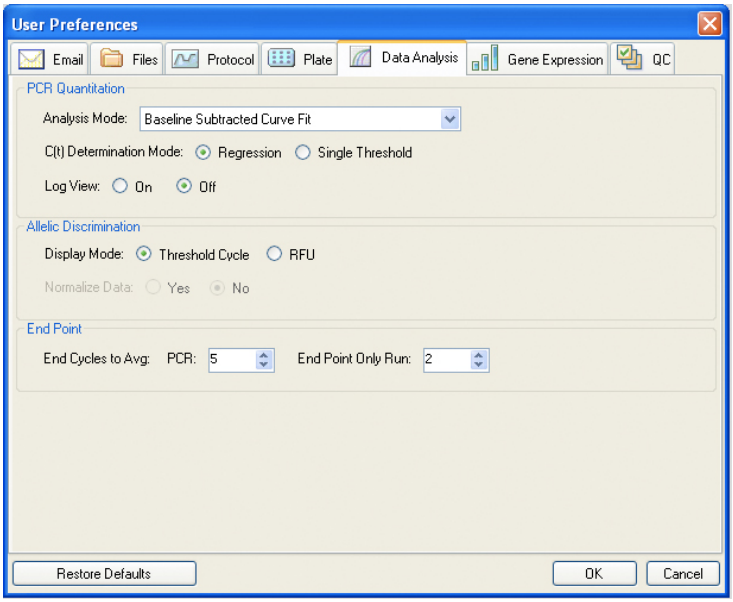


Figure 78. Data Analysis tab in the User Preferences window.

For the quantitation data, select the following settings:

- **Analysis Mode.** Select the default base lining method for the analysis mode. Choose Baseline Subtracted Curve Fit, No Baseline Subtraction, or Baseline Subtracted
- **C(t) Determination Mode.** Select between Regression mode or Single Threshold mode to determine how C(t) values are calculated for each fluorescence trace
- **Log View.** Select **On** to show a semi-logarithmic graph of the amplification data. Select **Off** to show a linear graph

For the allelic discrimination data, select the following settings:

- **Display Mode.** Select **RFU** to show the data as a graph of the RFU, or select **Threshold Cycle** to show a graph of threshold cycles
- **Normalize Data.** This selection is only available when RFU is selected. Select **No** to show non normalized data. Select **Yes** to normalize the data to the control sample

For end point data, select the following settings. Select the number of end cycles to average when calculating the end point calculations:

- **PCR.** Enter a number of cycles for PCR to average the end cycles for quantitation data (default is 5)
- **End Point Only Run.** Enter a number of cycles for End Point Only Run to average the end cycles for end point data (default is 2)

Gene Expression Tab

Select the **Gene Expression** tab in the User Preferences window to specify the default settings for a new Gene Expression data file:

- **Relative to.** Select a control or zero. To graph the gene expression data originating at 1 (relative to a control), select **Control**. When you assign a control sample in the

Experiment Setup window, the software automatically defaults to calculate the data relative to that control. Select **Relative to zero** to instruct the software to ignore the control, which is the default selection when no control sample is assigned in the Experiment Settings window

- **X-Axis.** Graph the Target or the Sample on the x-axis
- **Y-Axis.** Graph Linear, Log 2, or Log 10 scale on the y-axis
- **Scaling.** Select a scaling option for the graph. Leave the graph unscaled. Alternatively, choose a scaling option to scale to the Highest value or to the Lowest value
- **Method.** Set the default analysis mode, including normalized expression (ΔΔCt) or relative expression (ΔCt)
- **Error Bar.** Select Std Dev. for standard deviation, or Std. Error Mean for the standard error of the mean
- **Std Devs.** Select the standard deviation multiplier to graph the error bars. The default is 1. Change the multiplier to either 2 or 3

QC Tab

Select the **QC** tab in the User Preferences window to specify QC rules to apply to data in Data Analysis Module. The software validates the data against the enabled tests and the assigned values (page 96).

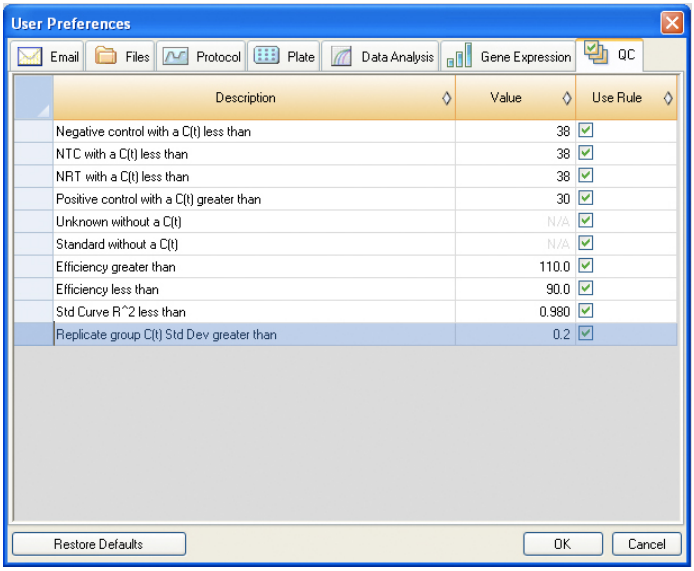


Figure 79. QC tab in User Preferences.

Specify to add cut off values and to enable the following QC rules:

- Negative control with a C(t) less than XX. Input a C(t) cut-off value
- NTC (no template control) with a C(t) less than XX. Input a C(t) cut-off value
- NRT (no reverse transcriptase control) with a C(t) less than XX. Input a C(t) cut-off value
- Positive control with a C(t) greater than XX. Input a C(t) cut-off value
- Unknown without a C(t)
- Standard without a C(t)
- Efficiency greater than XX. Input a reaction efficiency cutoff value that is calculated for the standard curve

- Efficiency less than XX. Input a reaction efficiency cutoff value that is calculated for the standard curve
- Std Curve R^2 less than XX. Input a cutoff R^2 value for the standard curve
- Replicate group C(t) Std Dev greater than XX. Input a cutoff standard deviation that is calculated for each replicate group

User Administration

Open the User Administration window in the main software window:

- Select **Users > User Administration**
- Click the **User Administration** button in the menu bar

If you log in as an Administrator, open the User Administration window to manage users and user rights:

- **Manage Users.** Add or remove Users, and assign each user a Role
- **Manage Rights.** Change rights for user roles (Principal, Operator, or Guest)

  NOTE: Only users who are Administrators can edit this window. Other users can only view it.

To assign a role to each user, select from the list of roles in the User Administration window (Figure 80). In this example, the Guest user is given the right to save files.

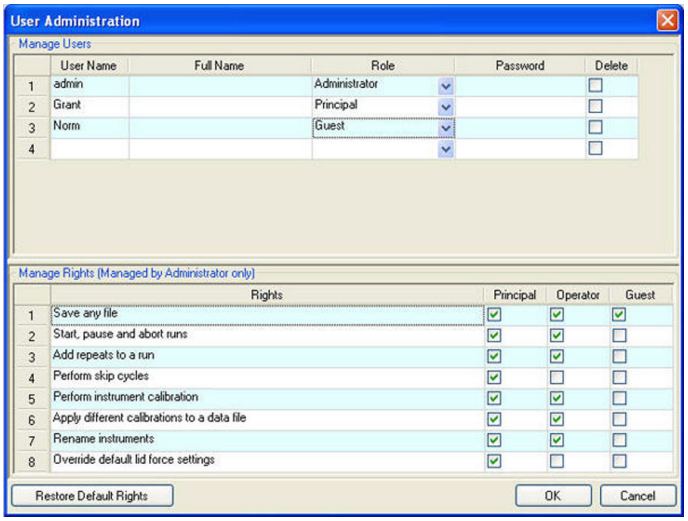


Figure 80. User Administration window with three users.

Adding and Removing Software Users

Only a software Administrator can add and remove users. To add software users in the Manage Users pane, follow these steps:

1. Enter a User Name for the new software user.
2. Select a user Role. These roles restrict the rights of each user. The default is Principal.
3. (Optional) Enter a Full Name and Password for the new software user.
4. Click **OK** to open a dialog box and confirm that you want to close the window.
5. Click **Yes** to close the dialog box and window.

To remove a software user, follow these steps:

1. In the Manage Users pane, click the box in the Delete list for each software user you want to remove.
2. Click **OK** to open a dialog box and confirm that you want to close the window.
3. Click **Yes** to close the dialog box and window.

   NOTE: The list of software users must always include one Administrator.

Assign Rights for User Roles

The User Administration window provides access to user roles and rights. The software includes these four roles:

- **Administrator (required).** Each Administrator has all rights, and you cannot change those rights. The Administrator can also add and remove software users and change the rights for each role
- **Principal.** By default, each Principal has all rights
- **Operator.** By default, each Operator has all rights except skipping cycles and creating a Gene Study
- **Guest.** By default, each Guest has no rights and can only read files

To specify the rights for each role, follow these steps. Only a software Administrator can change the rights for any role:

1. In the Manage Rights pane, click a box under the name of the role to add or remove that right. Click one or more rights in the list. To change all the rights for all the roles to the default list, click **Restore Default Rights**.
2. Click **OK** to open a dialog box and confirm that you want to close the window.
3. Click **Yes** to close the dialog box and window.

To view your current user role and rights, select **User > User Administration**. Contact a software administrator to modify the user settings, rights, and roles listed in the User Administration window. A Principal, Operator, or Guest user can view only their own user settings, rights, and roles.

11 Resources

Read this chapter to learn more about resources for the CFX system:

- LIMS Integration (page 99)
- Calibration Wizard (page 102)
- Instrument maintenance (page 104)
- Application log (page 106)
- Troubleshooting (page 106)
- References (page 108)

LIMS Integration

CFX Manager™ software can be configured for use with a laboratory information management system (LIMS). For LIMS integration, CFX Manager software requires plate setup information generated by the LIMS platform (a LIMS file, *.plrn), a protocol file created using CFX Manager software (*.prcl), a defined data export location, and a defined export format.

Creating a LIMS File

A LIMS file (*.plrn) contains the plate setup details and the protocol file name. CFX Manager software will use the LIMS file to create a plate file that will be used in conjunction with the named protocol file to start a run and generate data.

The following steps should be performed by a LIMS specialist.

1. Select the template file “CFX96 LIMS Plate Import Template.csv” located in the “SupportFiles” folder: [C:\Program Files\Bio-Rad\CFXIVD\SupportFiles\CFX 96 LIMS Plate Import Template.csv]. Use a text editor to edit template file.
2. Using the LIMS, complete the template by filling in the required fields as listed in Table 29.
3. Save the template with the file name extension .plrn directly to your LIMS file folder location. **Note: the .plrn files and the protocol files referenced in the .plrn files must exist in the same folder location.**

The .plrn file is a format known as a comma separated value (CSV) file. It utilizes the comma "," as the separator between fields. A comma should not be used within any field. Changing the file extension from .csv to .plrn is required for CFX Manager software to recognize the file and start a LIMS run.

Floating point numbers are parsed as US English, so the decimal mark used must be a period and not a comma. Also, for floating point numbers, the thousands separator should not be used.

Table 29. Definition of LIMS .csv file contents.

Column	Row	Description	Content	Purpose
A	1	Plate Header	Do not edit	Predefined
A,B,D	2	Field/Data/ Instruction	Do not edit	Predefined
B	3	Version	Do not edit	Predefined
B	4	Plate Size	Do not edit	Predefined
B	5	Plate Type	Enter “BR White”, “BR Clear”, or other calibrated plate type	Required
B	6	Scan Mode	Enter “SYBR/FAM Only”, “All Channels” or “FRET”	Required
B	7	Units	Enter one of the following: “copy number”, “fold dilution”, “micromoles”, “nanomoles”, “picomoles”, “femtomoles”, “attomoles”, “milligrams”, “micrograms”, “nanograms”, “picograms”, “femtograms”, “attograms”, or “percent”	Required
B	8	Run ID	Enter short description or barcode identifying this run. **Note:** Do not use commas in run ID	Optional
B	9	Run Note	Enter run description. **Note:** Do not use commas in run ID	Optional
B	10	Run Protocol	Protocol file name (protocol file and .plrn file must exist in same folder)	Required
A	11 & 12	Data File/TBD	Do not edit	Predefined
A	13	Plate Data	Do not edit	Predefined
A	14-110	Well Position	Do not edit	Predefined

Column	Row	Description	Content	Purpose
B-G	14-110	Ch1 Dye, Ch2 Dye, Ch3 Dye, Ch4 Dye, Ch5 Dye, FRET	Enter one calibrated dye name (for example “FAM”) for each channel being used	Required
H		Sample Type	Enter one of the following sample types: “Unknown”, “Standard”, “Positive Control”, “Negative Control”, “NTC”, or “NRT”	Required
I		Sample Name	Enter sample name	Optional
J-O	14-110	CH1 Target, CH2 Target, CH3 Target, CH4 Target, CH5 Target, FRET Target	Enter target name for each channel used	Optional
P		Biological Set Name	Enter biological set name	Optional
Q		Replicate	Enter a positive integer for each set of replicates. The value cannot be zero	Optional
R-W		CH1 Quantity, CH2 Quantity, CH3 Quantity, CH4 Quantity, CH5 Quantity, FRET Quantity	Enter quantity values for any standards. Enter concentration in decimal form	Required for all standards
X		Well Note	Enter well note	Optional
Y-AD		Ch1 Well Color, Ch2 Well Color, Ch3 Well Color, Ch4 Well Color, Ch5 Well Color, FRET Well Color	Enter any user-defined trace style color in a 32-bit integer (ARGB) decimal format	Not enabled

Initiating a LIMS Run

To initiate a LIMS run:

1. Open a LIMS file using one of the following methods:

- Drag and drop the .plrn file onto the CFX Manager software window or desktop icon
- Double-click on the desired .plrn file
- Select **File** > **Open** > **LIMS file** from the main software window menu bar

In the Experimental Setup Window, the Start Run tab is displayed. The Start Run tab includes a section for checking information about the run that is going to be started, including the selected protocol and plate file, and a section for selecting the instrument block.

Check the RUN Information. This information includes the protocol name, the plate name, and optional added notes.

2. Click the **Start Run** button to begin running the experiment on the selected block.

Calibration Wizard

The CFX system is factory-calibrated for commonly used fluorophores in white-well and clear-well plates (Table 30).

Table 30. Factory-calibrated fluorophores, channels, and instruments.

Fluorophore	Channel	Excitation (nm)	Emission (nm)
FAM, SYBR® Green I	1	450-490	515-530
VIC, HEX, CAL Fluor Gold 540, Cal Fluor Orange 560	2	515-535	560-580
ROX, Texas Red, CAL Fluor Red 610, TEX 615	3	560-590	610-650
CY5, Quasar 670	4	620-650	675-690
Quasar 705, Cy5.5	5	672-684	705-730

The CFX system also includes a channel dedicated for FRET chemistry; this channel does not require calibration for specific dyes.

To open the Calibration Wizard to calibrate the CFX system:

1. Select an instrument in the Detected Instruments pane.
2. Select **Tool > Calibration Wizard** to open the window and calibrate new dye and plate combinations (Figure 81).

Figure 81 shows an example of the Dye Calibration window.

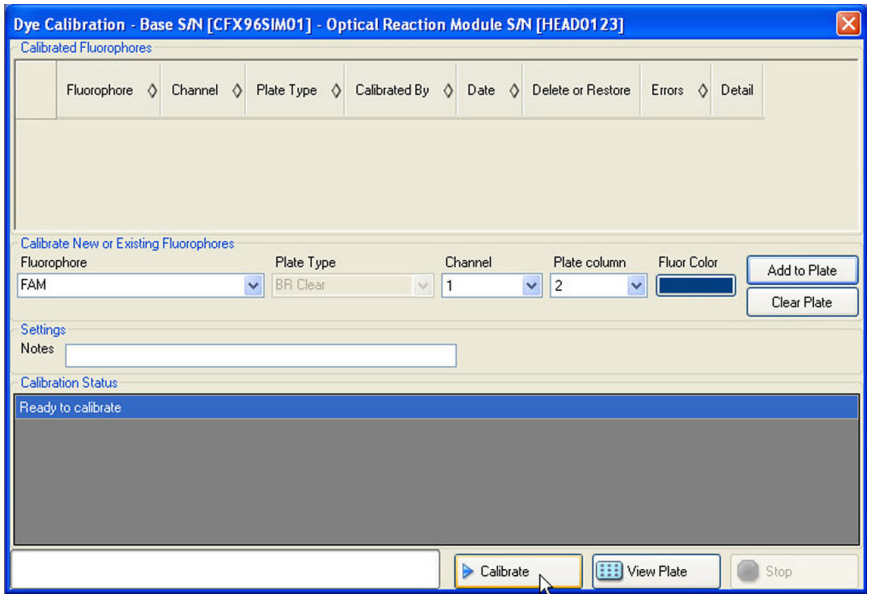


Figure 81. Dye Calibration window.

Calibrating the CFX System

To calibrate the CFX system in the Dye Calibration window:

1. In the Calibrate New or Existing Fluorophores pane, select the fluorophore you want to calibrate from the pull-down list. If the fluorophore name is not included in the list, type the name in the box to add it to the list.
2. Select the Plate Type. If the plate type is not included in the list, type the name in the box to add it to the list.
3. Select a Channel for the fluorophore.
4. Click **Add to Plate** to add the fluorophore. To clear the plate, click **Clear Plate** to remove all the fluorophores.
5. (Optional) Repeat steps 1–6 to add each fluorophore you plan to calibrate for the plate.
6. When you finish adding fluorophores, click **View Plate** to open the Dye Plate Display. Use this window as a guide for loading dyes into the plate.
7. Prepare a 96-well plate for dye calibration by pipetting dye solution into each well, following the pattern shown in the Pure Dye Plate Display. For each fluorophore, fill 4 wells with 50 µl (96-well plate) of 300 nM dye solution. Notice that at least half the plate contains blank wells.
8. Seal the plate using the sealing method you will use in your experiment.
9. Place the calibration plate in the block and close the lid. Then click **Calibrate**, and click **OK** to confirm that the plate is in the block.
10. When the CFX Manager™ software completes the calibration run, a dialog box appears. Click **Yes** to finish calibration and open the Dye Calibration Viewer.
11. Click **OK** to close the window.

Instrument Maintenance

The CFX system includes a sensitive optical shuttle system and a sample block that must heat and cool very fast. Contamination of these components can interfere with thermal cycling and data collection.

WARNING! Never allow a reaction to run with an open or leaking sample lid. The reagents could escape and coat the block, inner lid, and optical head in the shuttle system. Excessive dirt can dim the signal, and fluorescence contamination can create excessive background signal. Only trained Bio-Rad service engineers can clean the shuttle optical system.

Avoid contaminating the CFX system by following these suggestions:

- Always clean the outside of any container before placing it in the block
- Never run a reaction with a seal that is open, loose, punctured, or otherwise damaged; doing so could contaminate the block, inner lid, and optical system
- Never run a PCR or real-time PCR reaction with volatile reagents that could explode and contaminate the block, inner lid, and optical system
- Clean the block and inner lid periodically to prevent the buildup of dirt, biohazardous material, or fluorescent solutions (page 105)
- Never clean or otherwise touch the optical system behind the heater plate holes that are in the inner lid (Figure 82 on page 105)
- Clean the outer lid and C1000™ thermal cycler base on a regular schedule (for details see the C1000 thermal cycler instruction manual)

Cleaning the Optical Reaction Module

The block of the optical reaction and the C1000 thermal cycler base should be cleaned on a regular schedule to remove any debris or dirt that might interfere with proper function. Clean as soon as you discover debris and spilled liquids with a soft, lint-free cloth that is dampened with water. Cleaning the instrument allows precise instrument function. For more detailed information about cleaning the C1000 base, see the C1000 thermal cycler instruction manual.

WARNING! Never use cleaning solutions that are corrosive to aluminum. Avoid scratching the surface of the C1000 reaction module bay. Scratches and damage to this surface interfere with precise thermal control.

WARNING! Never pour water or other solutions in the C1000 reaction module bay. Wet components can cause electrical shock when the thermal cycler is plugged in.

Clean the CFX optical reaction module as soon as you discover debris, dirt, or contamination in the block or on the inner lid. Any dirt can interfere with the ability of the block to change temperature quickly and collect accurate fluorescent data. To clean the reaction module, follow these guidelines. Follow these suggestions for cleaning:

WARNING! To prevent electrical shock, always remove the reaction module from the thermal cycler base, or unplug the base before cleaning the instrument.

WARNING! Never touch or allow solutions to touch the optical system that is located behind the heater plate holes in the inner lid (Figure 82).

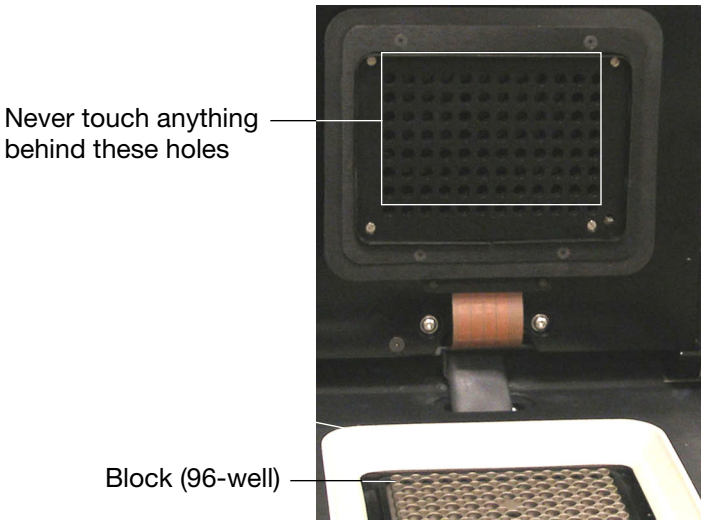


Figure 82. Heating plate holes in the inner lid.

- **Clean the outer surface.** Use a damp cloth or tissue to clean spills from the outside case. If needed, use a mild soap solution and rinse the surface with a damp cloth. Clean the cover to prevent corrosion
- **Clean the cooling fins.** Remove dust with a soft brush or damp cloth. Remove any heavy dust that is deep in the vents with a vacuum cleaner. Use water and a soft, lint-free cloth to remove debris that is stuck to the fins. Avoid scratching the surface. If needed, use a mild soap solution and rinse well to remove residue completely. Cleaning the fins improves precise sample heating and cooling

  NOTE: Never use cleaning solutions that are corrosive to aluminum such as bleach or abrasive cleansers.
- **Use of oil in the wells is not recommended.** If oil is used, the wells must be cleaned thoroughly and often. Remove the oil when it is discolored or contains dirt. Use a solution of 95% ethanol to clean oil. Do not allow oil to build up in the block.
- **Clean the wells in the block.** Clean spills immediately to prevent them from drying. Use disposable plastic pipets with water (recommended), 95% ethanol, or a 1:100 dilution of bleach in water. Also use a soft, lint-free cloth or paper towel and water to clean the block. Always rinse the wells with water several times to remove all traces of cleaning reagents

  WARNING! Never clean the block with strong alkaline solutions (strong soap, ammonia, or concentrated bleach). Never use corrosive or abrasive cleaning solutions. These cleaning agents can damage the block and prevent precise thermal control.

  WARNING! Bleach, ethanol, or soap that is left in the blocks could corrode the block and destroy plastics during a run. After cleaning, always rinse the wells thoroughly with water to remove all traces of cleaning reagents.

  WARNING! Never heat the block after adding a cleaning solution. Heating the block with cleaning solution will damage the block, reaction module, and thermal cycler base.

- **Clean the inner lid.** Use a soft, lint-free cloth and water to remove debris and solutions from the inner lid surface. Never use abrasive detergents or rough material that will scratch the surface. Cleaning the inner lid improves precise sample heating and cooling.

Application Log

Before a new run, the instrument initiates a self-diagnostic test to verify that it is running within the specifications. The software records results of this test in the Run log and Application log file. If you notice a problem in one or more experiments, open the run and application logs to find out when the problem started.

CFX Manager software tracks information about the state of an instrument during a run in the **Application Log** (Figure 83). Use these logs to track events that occur on instruments and in the software and for troubleshooting.

To open the Application log in the main software window, select **View > Application Log**.

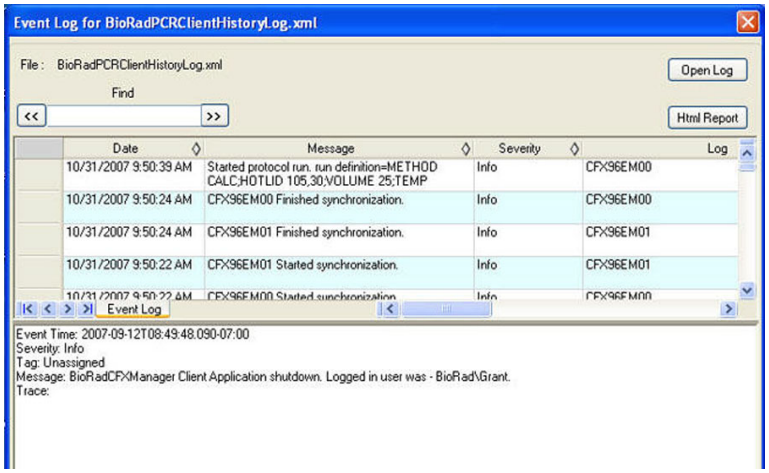


Figure 83. Example of an Event Log file.

Troubleshooting

Typically, software and instrument communication problems can be resolved by restarting your computer and the system. Be sure to save any work in progress before restarting.

NOTE: Check that your computer has sufficient RAM and free hard drive space. The minimum RAM is 2 GB, and the minimum hard drive space is 20 GB.

Installing the Software Manually

If needed, install the software manually by following these instructions:

1. Insert the software CD.
2. Right-click the software CD icon, and select **Explore** to open the CD window.
3. Double-click the **CFX_Manager** folder to open the folder, and then double-click **setup.exe** to start the software installation wizard.
4. Follow the instructions on the wizard to install the software, and then click **Finish**.

Power Failure Options

In a power failure, the instrument and computer will shut down. After a short power failure, the instrument will resume running a protocol, but the Application log will note the power failure. Depending on the computer settings and the length of time that the power is off, the instrument and software attempt to continue running depending on the protocol step:

- If the protocol is in a step with no plate read, then the protocol continues running as soon as the instrument gets power again
- If the protocol is in a step with a plate read, then the instrument waits for the software to restart and resume communication to collect the data. In this situation, the protocol only continues if the software is not shut down by the computer. When the computer and software start up again, the protocol continues

If you want to open a locked motorized lid on a reaction module to remove your samples during a power failure, follow these steps to remove the locking plate:

1. Remove the reaction module from the C1000 chassis by pushing down on the locking bar of the C1000 base.
2. Place the module on the front of a desk, so that the front of the module extends 2 inches over the edge of the desk as shown in Figure 84.

Figure 84. Setting up the Optical Module to remove the locking plate.

3. With an Allen wrench, remove the two large screws from under the front edge of the reaction module (below the button for opening the lid). Do not remove the two small screws that are located at the front edge of the module. You should hear the locking latch release from inside the module. Figure 85 shows the two large screws.

Figure 85. Remove these screws to unlock the optical module.

4. Push the reaction module lid open. Notice that the latch (dark plastic) is no longer attached. Remove your samples from the block.
5. Reassemble the reaction module with the lid open by replacing the locking latch and securing it with the large screws. Figure 86 shows the locking latch in place.

Figure 86. Optical module locking latch.

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